LIBRARY
University of Montana

THE
AMERICAN NATURALIST

VOL. LXVI *March–April, 1932* No. 703

THE AMERICAN SOCIETY OF NATURALISTS

SEX-INHERITANCE AND SEX-DETERMINATION[1]

PROFESSOR CHARLES E. ALLEN
UNIVERSITY OF WISCONSIN

IN what follows, sex will be considered as implying the existence of certain demonstrable structural and functional differences. These differences distinguish complementary adaptations to needs imposed by the occurrence of gametic union, and by the increasing length of an embryonic period for which provision must be made by the parental or (in angiosperms) the grandparental generation. In some current biological discussions sex is understood more broadly than as here defined. Reference is unnecessary to more weird conceptions entertained by the uninformed—say, by post-war novelists or by literary M.D.'s.

Sexual differentiation has evidently developed independently in many distinct lines of descent. The algae, particularly the Isokontae, furnish the most extensive evidence at hand of the course of its evolution. As yet, however, the algae have revealed little regarding the determination and inheritance of sexual characters. So far as plants are concerned, it is necessary to turn to two other groups—bryophytes and angiosperms—for any extensive light upon these questions.

Any genetic analysis of sex in angiosperms must deal almost exclusively with characters of the so-called

[1] Paper read at the symposium on "The Biology of Sex" before the American Society of Naturalists, New Orleans, December 31, 1931.

asexual generation, since those of the much-reduced haploid "sexual" generation have yet afforded little material for genetic study. To speak of sexual characters in an asexual generation is paradoxical; but the paradox inheres in the terminology, not in the facts. The diploid sporophyte helps through various devices to effect the union of gametes produced by the filial gametophytes, and to provide for the shelter and nutrition of the embryonic grandfilial sporophyte; and such devices are sexual characters under any usable definition of the term.

This differentiation in the sporophytic generation is very different in its phylogenetic implications from sexual differentiation in any metazoon. Functionally, however, the same ends are subserved; and, somewhat remarkably, the genetic mechanisms that govern in the two cases are strikingly similar.

Many pages of discussion of the present and related problems would not have burdened the pages of scientific journals had their writers appreciated two fairly obvious facts: first, that the potentiality for the production of any character that an organism under any possible conditions can manifest must be represented in its hereditary constitution; and second, that this hereditary constitution provides *only* potentialities, whose expression or non-expression depends upon the concurrence of environmental factors.

The question then presents itself: What potentialities for sexual differentiation exist in an angiosperm? In a large majority of species, each plant, under anything approaching ordinary environmental conditions, regularly produces both stamens and pistils—as well as, often, intersexual structures. For such bisexual organisms the answer to the question is, clearly, that both male and female potentialities are represented in the hereditary substance. On the other hand, in a minority of species some plants produce only or chiefly staminate flowers, other plants only or chiefly pistillate flowers. It

is mainly with these dioecious species that discussions of sex-determination in angiosperms have dealt.

In most instances in which the floral structures of dioecious, or so-called dioecious, angiosperms have been extensively studied, it has been found that a pistillate plant now and then bears a staminate or bisexual flower and that a staminate plant occasionally produces a pistillate or bisexual flower. Sometimes intermediately bisexual plants occur in addition to those which are strictly or chiefly male or female. Such conditions characterize, for example, dioecious species of nettle, hop, hemp and spinach. The closest approach to an absolute separation of sexual characters seems to be shown in *Lychnis dioica, L. alba* and *Bryonia dioica.* But even for *Bryonia,* there is one report of the occurrence of occasional staminate flowers on a female plant. In both dioecious species of *Lychnis* hermaphroditic plants are described which are shown, in one case by genetic, in the other by cytological, evidence to be modified males. In both species, too, it has long been known that infection by the anther smut induces the development of stamens by a female plant. The evidence, therefore, is overwhelming that in dioecious as in hermaphroditic angiosperms each plant possesses both male and female potentialities. The possibility of course remains, though present evidence opposes it, that strains or even species may be found with more limited genetic capacities.

It is generally agreed that nothing points directly to specific genes responsible for sexual potentialities. But these must be represented by some basis or bases in the hereditary mechanism. Anglicizing a term already much used, such bases may be termed, respectively, male- and female-potency factors. There is no reason to suspect that these factors are borne by X and Y chromosomes, even in those species in which such allosomes are recognized. Since every plant is endowed with male and female potentialities, the factors basic thereto must be so located that for each the plant is homozygous. This

fact excludes from consideration the Y chromosome, and, though perhaps not so certainly, the X; moreover, as will appear, the X–Y mechanism is otherwise especially concerned. So it may be held as probable that the sex-potency factors in all angiosperms are connected with one or more pairs of autosomes.

A further explanation is needed for the one-sided type of sex expression found in dioecious species. Here, evidently, factors are involved which, superposed as it were upon those just mentioned, favor the appearance of one set of sexual characters and tend to inhibit that of the other set. Factors of this superposed set have been referred to as sex-*determining*. However, since this term may irk some of our colleagues who are strongly impressed with the determining effects of the environment, it is more discreet to speak of male and female *tendencies*. An important contrast appears between sex-potency and sex-tendency factors; namely, that, whereas both (or both sets) of the former exist, each in a homozygous condition, in every plant, on the otner hand, with respect to the factors for sex tendencies a condition of heterozygosis must prevail in one sex.

The recognition of a distinction between sex potencies and sex tendencies in angiosperms is due to Correns. The former he considers as represented by gene-complexes, A and G; the latter, by genes or gene-complexes, α' and γ'.

Omitting questionable cases, X and Y chromosomes are known in some 45 species, representing 19 genera, of dioecious angiosperms. The distribution of these chromosomes parallels that of the sex tendencies. The known facts fit an assumption that the female-tendency factor is located on the X chromosome, the male-tendency factor on the Y. It is quite possible, to be sure, that angiosperms may be found in which the female rather than the male is heterozygous with respect to these factors. That this is the condition in certain dioecious strawberries is strongly indicated by experimental and cytolog-

ical studies. In 26 dioecious species, belonging to 22 genera, the search for unlike chromosome pairs has been unsuccessful. Since the study has been limited for the most part to the microspore mother cells, it may be that the females of some of these species possess an X–Y pair.

Should species be found with an X and no Y (a condition not yet definitely known for an angiosperm), the assumption just noted will require modification, at least for those species, by supposing that the male- (or female-) tendency factor is borne on an autosome. Such a conception would be very close to that advanced by Bridges for *Drosophila,* with the added feature of a recognition of the distinction between sex potencies and sex tendencies.

It may not be comely for a botanist to express opinions bearing upon conditions in animals. However, since zoological writers have shown neither undue modesty nor excessive caution in treating of botanical phenomena, one suggestion will be ventured. Correns' analysis has not found favor among animal geneticists. But wherever hermaphroditism, intersexuality or sex-reversal occurs—and these are now recognized as wide-spread phenomena in several metazoan phyla—the potentialities for the production of the characters of both sexes must reside in each individual. The more or less sharp differentiation of male and female individuals, the genetics of sex and the occurrence of the X–Y chromosome mechanism, all parallel to conditions noted in angiosperms, evidence the presence of sex tendencies superposed upon a duplex set of potentialities.

Sex expression in angiosperms as in other organisms is of course influenced by many genetic factors apart from those mentioned. These others, diverse though they be, may be grouped together for present purposes as sex-*influencing* factors. Among them are those which affect, very differently in different species, the numbers, succession and arrangement of stamens and pistils; or which bring about such modifications of strict hermaph-

roditism as monoecism, andromonoecism and gynomonoecism; or conditions intermediate between hermaphroditism and dioecism, like andro- and gynodioecism. The class would include those factors experimentally studied in maize, which influence the proportions, distribution and functioning of staminate and pistillate flowers.

Admittedly, it may not always be easy to distinguish between factors of this class and the sex-tendency factors or mutants thereof. For example, Shull concludes that the appearance of hermaphroditic *Lychnis* plants, shown to be modified males, results from a mutation of the male-tendency factor; but the very comparable occurrence of intersexes in *Drosophila simulans* is found by Sturtevant to be caused by a recessive gene located on an autosome. As another illustration, the action of certain of the factors in maize just mentioned may result in the appearance of purely female plants, thus simulating the effect of a female-tendency factor.

Environmental factors likewise produce effects comparable with those of the sex-tendency factors, inhibiting the expression of one set of sexual characters and favoring the expression of the characters of the opposite sex. An environmental factor, as nutrient or light, may thus partially or completely reverse the effects produced in the environment usual for the species by the genetic sex-tendency factors. This possibility is abundantly shown by the work of Schaffner among many others. Environmental factors also often produce effects parallel to those of the sex-influencing factors; but never anything of the order of the effects of the sex-potency factors. These latter endow the plant with both sets of sexual possibilities, and the possibilities remain unchanged, however their expression may be encouraged or discouraged by other factors genetic or external.

In considering sexual conditions in bryophytes, it must be remembered that the plants here in question belong to a haploid generation. This generation corresponds,

so far as there is correspondence, to the angiosperm gametophyte, regarding whose mechanism of sex-determination nothing is known. Another important consideration is that any relationship between bryophytes and angiosperms is at best extremely remote; possibly, indeed, they are climax members of distinct evolutionary series derived from separate protistan ancestors. It would not be surprising, then, should the mechanism of sex-determination prove to be radically different in the two groups. It would be less surprising, indeed, than was the finding of a similarity in this regard between angiosperms and insects.

The bryophytes, to be sure, show a distribution of sexual characters superficially like that found in angiosperms. Both groups include hermaphroditic and dioecious species. Whereas a majority of angiosperms are hermaphroditic, among bryophytes, to judge from taxonomic descriptions, a majority, though not an overwhelming one, are dioecious. But with respect to this point, more or less casual field and herbarium observations of limited numbers of plants must be checked by extensive observation and experiment.

Numerous sex-influencing factors are present in bryophytes, as shown by the fact, among others, of the occurrence of specific differences in the distribution of sexual organs. At least seven distinct racial characters are now known in Sphaerocarpos, each of which involves the structure or functioning, or both, of sexual organs. Environmental factors also produce comparable effects. The appearance of the sexual structures of *Marchantia* can be inhibited by conditions of illumination. Nutritive conditions are shown to determine, in some measure, the production of male or of female organs by *Funaria*. But environmental conditions have not been found able to reverse the sex expression of a dioecious bryophyte, as they can reverse that of a dioecious angiosperm.

Extensively studied thus far are a few dioecious mosses, including those involved in the classic experi-

ments of the Marchals; and a few dioecious hepatics, among them the familiar *Marchantia polymorpha* and two species of *Sphaerocarpos.* These studies agree in showing that strict unisexuality exists; that is, any haploid plant of such a species is destined from the spore to be either male or female, and no change in conditions, applied at any stage, has induced intersexuality or sex-reversal. Beside this fact must be placed another, namely, that a single genus, such as *Marchantia,* may include hermaphroditic and strictly dioecious species. There are suggestions of the possible existence of an intermediate condition—one of potential bisexuality combined with a tendency toward dioecism. But in no species thoroughly studied has this condition been found; and the possibility must for the present be ignored.

In at least five dioecious hepatics, X and Y chromosomes are certainly recognized; one X being present in the female, one Y in the male. In others, including *Marchantia,* and in dioecious mosses in general, no such allosomes have been distinguished. In these latter cases, nevertheless, the phenomena of sex expression are the same as in species known to possess sex chromosomes.

Polyploid gametophytes of normally dioecious mosses have been obtained by experimental methods—most extensively by the Marchals, Schweizer and Wettstein. A diploid gametophyte containing two maternal genoms is female; one with two paternal genoms is male. A diploid possessing one maternal and one paternal genom, or a tetraploid with two maternal and two paternal genoms, is bisexual. A triploid with two maternal genoms and one paternal genom is also bisexual, but with the female tendency more strongly marked than in the bisexual diploid. An example among hepatics is the diploid bisexual clone of a normally dioecious *Pellia* studied by Showalter.

A somewhat different case is presented by *Sphaerocarpos.* In the capsules of those races whose spores are

regularly adherent in tetrads, spore dyads occasionally appear. Lorbeer found that the spores of such dyads give rise to females. Cytological study showed these females to be diploid, each possessing an X and a Y chromosome—having, that is, the chromosome complement characteristic of the sporophyte. I am able to corroborate Lorbeer's statements that the spores of dyads give rise to clones that are apparently female and that possess the diploid chromosome complement—although the possibility of an occasional deviation from exact diploidy is not yet excluded. Twelve such diploid clones have produced sporophytes in matings with haploid males—thus showing themselves functionally female. However, in sections of two of the diploid gametophytes, a few organs have been found similar to the intersexual structures described by several workers in hermaphroditic mosses. No such organs have been found in haploid plants of Sphaerocarpos. It appears probable, then, that the diploid clones are really bisexual, as are corresponding diploids in the other cases mentioned—although in Sphaerocarpos the female tendency almost completely dominates the male. Thus the results with Sphaerocarpos seem to agree with those derived from other dioecious bryophytes in showing that only one set of sex possibilities is represented in each haploid chromosome complement, and that evidence of bisexuality appears when both maternal and paternal complements are present.

Correns has applied to the analysis of the sexual conditions of bryophytes the same formula that he developed for angiosperms. He assumes the presence in both hermaphroditic and dioecious bryophytes of the genes (or gene complexes) A and G (the sex-potency factors), and in dioecious species of the additional genes α and γ (the sex-tendency factors). The presence of A and G in a dioecious species implies that each plant of the species possesses both male and female potentialities. But to this conclusion, inevitable for dioecious angiosperms,

dioecious bryophytes thus far afford no support. On the contrary, all the known facts indicate that a haploid male plant of such a species is and can be only male, and that a haploid female plant is and can be only female.

It is unnecessary, therefore, to assume that a dioecious bryophyte possesses distinct sex potencies and sex tendencies. Apart from sex-influencing factors, only one set is needed to explain the sexual conditions. This set might be likened to both the sex-potency and the sex-tendency factors of angiosperms. Perhaps, since homology is out of the question, and since genetic sex-determination here is so much more effective than in angiosperms, it may be allowable to speak of sex-*determining* factors.

In dioecious bryophytes with recognizable X and Y chromosomes, the sex-determining factors can hardly be thought of otherwise than as borne on these allosomes. Sex behaves in inheritance in a unitary fashion; it must be considered, then, as determined by a pair of factors or of closely linked factor-complexes. It would seem that the female determiner must be borne on the X chromosome. The male determiner being a positive thing, since its presence with the female determiner (in diploids) induces bisexuality, it is naturally thought of as borne on the Y. A hypothesis could be devised according to which a male-determiner is borne on an autosome, and its tendency, much as in Bridges' theory, is overborne by that of a female-determiner on the X. But such a theory is needlessly complicated for the facts in the bryophytes as now known, and, indeed, would not fit those facts so well as the simpler formula here proposed.

The formula is easily applied likewise to hermaphroditic species. In these, each plant has a male-determining and a female-determining factor, the two being borne on the same or on different chromosomes. If on the same chromosome, it can be imagined that in the sporophyte, where that chromosome meets its homologue, by loss or by interchange one chromosome of the pair loses

its female-, the other its male-determiner. Thereafter, half the gametophytic offspring would be male, the other half female—a dioecious race thus arising.

Although the evidence from varied sources indicates that hermaphroditism is the more primitive and dioecism a derived condition—its derivation in bryophytes possibly to be accounted for in the way just suggested—the probability is strong that hermaphroditic have in turn originated secondarily from dioecious species. Essentially this has happened repeatedly under observation whenever, by experimental means or otherwise, a diploid plant was produced which possessed the two sex-determiners. Heitz's observation that hepatics with 8 or 9 chromosomes are predominantly dioecious, while those with 16, 18 or other multiples of the basic number are predominantly hermaphroditic, suggests that hermaphroditic species have from time to time thus originated in nature.

The scheme suggested for bryophytes, then, involves a single female-determining and a single male-determining factor. Both are present in the chromosome complement of a haploid hermaphroditic species. In a dioecious species, each plant has only the female-determiner, borne on an X chromosome, or the male-determiner, borne on a Y chromosome. Diploid hermaphroditic species possesses both the X with a female determiner and the Y with a male determiner. It is judicious to anticipate the suggestion that discoveries may be announced to-morrow which will render this simple analysis inadequate. It fits the facts as they are known to-day.

PHYSIOLOGY OF EMBRYONIC SEX DIFFERENTIATION[1]

DR. EMIL WITSCHI
STATE UNIVERSITY OF IOWA

LAST summer I heard one of our most prominent geneticists sum up past and future trends in developmental research with a rather striking parable. The geneticists have been searching Olympus, he said, for the gods which the embryologists were disrespecting on earth; what both ought to do, however, is to trace the hands of God in the works of nature. I confess that it sounded quite agreeably when genetics was leveled up to theology. Some of us will remember the time, only a few years past, when efforts to approach sex determination from a physiological as well as from the genetical point of view met with derision because they seemed to undervalue one of the most triumphant of recent biological discoveries and to exhibit the lack of a clean-cut conviction. That was when one X was equal to male and two X were equal to female. Though time has kept changing, McClung, first to point at the apparent correlation between odd chromosomes and sex determination (1902) was first again to propose a physiological interpretation based on the cytological peculiarities of the X-chromosome in spermatogenesis (1918). Taking up his suggestion, the following facts may be brought to light.

In the spermatogenesis of many grasshoppers, especially in orthopterans, the single X displays some odd features, such as heteropycnosis in interkinetic stages and lagging during mitosis. None of these peculiarities is exhibited in ovogenesis, which seems to suggest that the X is physiologically inactive in spermatogenesis.

[1] Paper read at the symposium on "The Biology of Sex" before the American Society of Naturalists, New Orleans, December 31, 1931.

This assumption agrees well with the result of genetical analysis, showing X as the carrier of the female determining gene. It seems significant again that even in spermatogenesis the X behaves normally up to the stage of the second spermatogonia. Before this, male and female germ cells are morphologically of the same type, and in cases of sex reversal it has been found that up to that stage they are capable of developing either into eggs or into sperms. The peculiarities of the X therefore start at the time of the final sexualization of the individual cell. At this point now arises the question whether they are the cause or merely a consequence of this sexualization. The alternative, in my belief, has to be answered in the second sense.

We shall not enter into a discussion of the fact that the sex chromosomes behave normally in both sexes of the amphibians and of other animal groups—as shown by the cytological studies of Stohler and Makino on toads and my own on the frog. We will only briefly consider the illuminating case of the stone fly, *Perla marginata,* where in the male a rudimentary ovary is found attached to the testes. Junker has made a cytological investigation of these rudimentary hermaphrodites and reports that the haploid X chromosomes show heteropycnosis in the spermatocytes, while in ovocytes they become attenuated like the diploid autosomes. This striking difference in the behavior of the X chromosome in ovo- and spermatogenesis seems to indicate that its odd features are a consequence only and not the cause of sex differentiation. We admit that observational facts alone will never furnish binding proof of causal connections. None the less, the presented case is fit to demonstrate that the sex chromosomes give not a solution to the problem of sex determination but rather represent one of its most intricate implications. Moreover, the fact that male and female differentiation proceeds here on an identical chromosomal basis proves that the sex genes are no absolute rulers. Cognizance of their flexibility will en-

courage the experimentalist. The only way to get an insight into the mechanisms of nature is by imitation in experimental settings. A study of the mechanism of embryonic sex differentiation has, therefore, to be based on experimental sex determination.

Unfortunately many workers in this field have had no further aim than to demonstrate the feasibility of artificial sex determination or of sex reversal. The arrangement of their experiments is not so planned as to provide much insight into the physiological principles involved. This is especially true for most of the plant work. Schaffner, Yampolsky and others have been quite successful in upsetting Mendelian sex ratios in diverse dioecious plants. One may generalize that they obtain their results by modifying, directly or indirectly, the nutritional conditions. Many, though not all, conform to Goebel's rule that the male sex organs are formed under nutritional conditions, which are insufficient for the production of female ones. This covers also the interesting observations of Cobb-Steiner and Caullery on some parasitic nematodes which are female in single infestations but male if living in large numbers within the same host. However, general starvation has proved unyielding as a means of male production—proving that specific trophic factors and not the total food quantum are concerned. Joyet-Lavergne in his physico-chemical theory of sex tries to account for this with his "second law of sexualization." He contends that differences in nature and amount of lipoid and fat reserves constitute a fundamental and primary character of sex.

Female cells are accumulating reserves of fats. The French author is somewhat contradictory about the question whether or not some of these lipoids are exclusively found in the female. It is evident that the so-called law largely describes only the well-known facts of gametic sex dimorphism. We are interested, therefore, to see how it stands the proof at critical points. How does it meet, for instance, the facts of relative sexuality of lower

organisms? Max Hartmann and others were able to show that in isogametic thallophytes so called "weak males" and "weak females" are capable of functioning either as males or as females, according to the type of gametes with which they happen to come in contact. Evidently this relative sexuality excludes the assumption of specific lipoids, essential for the female sex.

It now remains to test the theory of characteristic proportions. Let us put the case of the Drosophila fly on trial. We can take for granted that the ovocytes have a higher fat content than the spermatocytes. Since the two types of sperms produce their well-known sex-determining effects the theory compels us to assume also that the X sperm has a relatively higher fat content than the Y sperm. However, we know through the non-disjunction work of Bridges that this X sperm determines a male zygote if it happens to fuse with an exceptional egg without X. Any general theory based on sex-determining cytoplasmic inclusions will be defeated at this point by the clear-cut facts established by chromosome research.

We can consider only briefly the metabolic theory, which is the objective of Joyet-Lavergne's "first law" and is best known through the extensive work on pigeons by Riddle. It says that the oxidation-reduction potential in a given species shows a lower value in the female than in the male. This again is the proper summary expression of a vast descriptive material as reported by Benedict, Dubois and others for man, Riddle for pigeons, Gayda for the toad, Hyman for Hydra, and so on. But experimental evidence of a causal relationship between metabolic level and sex differentiation is entirely wanting. In fact some experiments much rather point to the opposite. So the famous case of the Echiurid worm Bonellia. Since Spengel, we know that larvae settling down on the proboscis of some female develop into dwarf males. Baltzer was able to prepare extracts of female tissues which caused the same masculinization.

Recently Herbst succeeded in inducing an identical reaction simply by increasing or decreasing the hydrogen-ion concentration. He interprets this result as a consequence of a decreased respiration rate following any deviation from the optimal pH of normal sea-water.

My own temperature experiments with frogs have often been claimed to sustain the metabolic theory. Under the condition of a maximally raised temperature the ovaries of larval females transform into testes. If, however, an accelerated metabolism in itself were instrumental in male determination, we would have to expect some effect in this direction as soon as temperatures rise even slightly above normal. In fact, sex reversal does not start until we reach the point where the increase of temperature does not further accelerate the total metabolism, but gives it a distinctly catabolic character. Similar considerations apply to the male-determining effect of uterine overripeness of frog eggs. It would rather appear that an insufficiently low or an abnormal metabolism is somehow related to the masculinizing effect.

Differences in osmotic pressures, electrical potentials and hydrogen-ion concentrations have further been suggested as primary factors of sexualization. All these schemes suffer the same mistake as the metabolic theory, that is, to deny sex its own fundamental character. Sex certainly involves metabolic processes which sometimes may find a more exact expression in the readings of respiration manometers or potentiometers than in the delicate morphological changes which they entrain. However, we can not see that the more specific and basic properties of sex could possibly be expressed in terms of metabolism.

In a general theory of sex one would have to include the results of genetics and cytology as well as those of experimental embryology. The amphibians lend themselves favorably for research in problems of sex just because they can successfully be studied from either of these three points of view. The genetic and chromo-

somal analysis has disclosed that two genes are involved in the hereditary transmission; namely, a male factor which is localized in the autosome group and a female factor which lies in the sex chromosomes. The genetical sex formula resembles therefore that of Drosophila, save for the presence of a female factor (f) in the Y chromosome. The discovery in 1914 of this quantitative allelomorph of (F) was the first evidence of a gene localized in a Y-chromosome. In short, the genetic constitution is always *bisexual,* and a chromosome mechanism distributes the female genes in such a way that one half of the zygotes receives a relatively higher quantity than the other. The two classes are conventionally distinguished as genetical females and genetical males. Though, let us for once consider them more critically.

There are some local races of frogs in which the so-called males have ovaries all through their early development. They transform into testes only during the second half of the first year. In the same localities old females very often transform into males. We see here that the sex is not only a function of the relative gene quantity but also of age. Again, if we consider other local races which under average conditions always show the expected 1:1 ratio of males and females, we find that the "genetical males" develop ovaries at low temperatures while the "genetical females" transform into actual males at high temperatures. Therefore, sex appears this time as a function of genetic balance and temperature. For the interpretation of this double determination it is essential that breeding experiments with sex-reversed females show the original genic basis unaltered. Consequently, the interference of such factors as age or temperature must enter the play somewhere along the course of events that run between the genes and their final phenotypic manifestation.

I thought that this might give us a means to find out something about the working ways of the genes. While studying the effect of low temperature on the process of

sex differentiation in genetical males, I soon realized that the development of the medullar cords of the gonads was much more retarded than that of the cortex. At the same time ovocytes were beginning to form in the cortex, as soon as it reached a certain size, regardless of the genetical sex of the animal. Later, when the medulla had attained a larger size, spermatogonia were formed. Therefore, germ-cells of identical constitution differentiate in the female or the male sense according to their relation to cortex or medulla. Observations of this type led to the conclusion that cortex and medulla play a rôle of inductors, respectively. A nature experiment discloses a further interesting characteristic of these inductors. The anterior part of the sex glands of the toads consists of cortex only. In accordance with the theory just submitted it always differentiates into an ovarial lobe, in the male as well as in the female. Of special interest, however, is the fact that during the period of early sex differentiation the anterior lobe develops much faster than the posterior part of the gonad. The delay in this region where the functional ovaries and testes form, is clearly caused by a struggle for dominance between cortex and medulla. We realize, then, that each inductor is not only stimulating the differentiation of one sex, but it also tends to suppress its opposite. Cortex and medulla form an antagonistic pair of inductors. Their play is possibly best uncovered by the high temperature experiment. Young females transferred from normal to high temperature suffer degeneration of the ovarial cortex. Soon after, the medullar cords start a compensatory hypertrophy which ends with a perfect transformation of the ovary into a testis.

Sex determination offers now the following aspect. Female and male genes control the activation of cortex and medulla, that is, the female and male inductor systems. Dominance is not decided by gene quantities directly, but is the outcome of the competitive efforts of the antagonistic inductors to gain control. An asym-

metrical quantitative distribution of the female genes through the sex chromosome mechanism may regulate the final sex determination under certain conditions. In general, however, environmental factors, favoring or disturbing selectively the development of the inductor systems, help to determine the course of the embryonic differentiation.

From the foothold thus gained, it is possible to explore further into the field of developmental physiology. We have especially been interested in the process of induction. Burns was first to apply Born's technique of amphibian parabiosis in sex research. Humphrey and myself, with a number of my students, have contributed in accumulating data that give now a well-rounded picture of some phases of the inductive process.

Embryos of frogs or of salamanders were grafted together so as to form twin pairs, long before the stage of sexual differentiation. According to the principles of chance combination, it is legitimate to expect that half of the pairs consist of a genetical male and a genetical female. In the case of salamanders and newts the male twin generally develops normal testes, while the ovaries of the female, after some initial differentiation, are reduced in size and finally become nearly or completely sterile. In exceptional cases, if the ovaries of the female get a sufficiently early start, the reaction appears reversed. We speak of a "free-martin reaction," because the effect is much the same as described by Lillie for chorionic twins in cattle. The same interpretation certainly will serve both the urodele and the cattle case. Cortex and medulla effect their inductions by releasing specific hormones, each interfering with the normal development of the other. If the two hormones appear at about the same time, the male one soon puts the female inductor out of action. Female dominance is only established if the ovaries attain a considerable size before the testes become differentiated at all.

The same experiment gives a somewhat different result with frogs. Here, ovaries and testes both develop unimpaired, if they are located far enough apart. In case they come closer together, those parts of the ovaries that lie nearest to the testes suffer first the arrest of development. Altogether, we notice here that the inductive effect spreads with a falling gradient, a mode which resembled more the type of embryonic induction which became known through the work of the Spemann school. As in the urodele twins, induction goes out from both ovaries and testes, male induction finally taking the lead in all cases.

The same experiment a third time repeated with toads does not reveal any signs of inductive interference. Induction apparently is confined within the sex glands. One is tempted to speak of an ascidian type, since Conklin established the lack of inductive transmission in Styela and Ciona.

So far I have been speaking of inhibitory induction only, since this is best worked out now. Evidence of stimulative induction is accumulating, however, which gives proof of the same three types of transmission.

Induction, generally speaking, seems to be effected by substances which are specific with respect to origin, chemical nature and substratum on which they act. In the three types just described these substances are so much alike regarding their origin and their physiological properties that we must assume a close relationship. In other words, we feel justified now and even compelled to consider formative substances and hormones as belonging in one class. It is in agreement with this interpretation when Conklin finds that the formative substances of the ascidian blastomeres are not identical with mitochondria nor with any other visible cell inclusion. Successful experiments of Spemann and his collaborators to obtain morphogenetic induction with non-living débris and with extracts prepared from amphibian organizers

add support to the notion of a hormonic, *i.e.*, a relatively simple chemical nature of the inductive substances.

We have to pay tribute to the genius of Jacques Loeb, who, years ago, had already conceived this solution in general terms. We remember also that Goldschmidt gave Loeb's idea a central place in his "physiological theory of inheritance." Nor shall we forget, however, that we have barely started now to provide this theory with a substantial basis of experimental facts.

The physiology of embryonic differentiation has long been one of the cardinal problems in biology. Up to recent times development has either been considered as a creative process or else as a mere growth of preformed minute germs into visible proportions. The aspect has fundamentally changed when Mendelian genetics revealed the existence of a complicated implicit structure of the organism which is by far more durable and less modifiable than its ever-changing and in generations pulsating explicit form. How are genetical constitution and phenotypical manifestation linked together? In the approach of this great problem it appears that embryonic sex differentiation offers unique research possibilities. It is the field in which genetics and embryology have been most successful, so far, in the much-needed and much-endeavored cooperation.

SOME GENETIC ASPECTS OF SEX[1]

PROFESSOR H. J. MULLER
UNIVERSITY OF TEXAS

I. SEXUALITY

FROM the genetic point of view it is advantageous to begin by considering sex in the broader sense of sexuality. It is not generally realized that genetics has finally solved the age-old problem of the reason for the existence (*i.e.*, the function) of sexuality and sex, and that only geneticists can properly answer the question, "Is sex necessary?" There is no basic biological reason why reproduction, variation and evolution can not go on indefinitely without sexuality or sex; therefore, sex is not, in an absolute sense, a necessity, it is a "luxury." It is, however, highly desirable and useful, and so it becomes necessary in a relativistic sense, when our competitor-species also are endowed with sex, for sexless beings, although often at a temporary advantage, can not keep up the pace set by sexual beings in the evolutionary race and, when readjustments are called for, they must eventually lose out. Thus sexual beings form most of the central and the continuing portions of the evolutionary tree from which ever and again new sexless end-twigs sprout off.

Whatever the secondary needs of present-day somatoplasm may be, there is no fundamental protoplasmic need for rejuvenation of the germ plasm through sexual union, no reason to believe that "protoplasmic stimulation" is *per se* produced by mingling of unlike germ plasms, nor any evidence that variation of the hereditary particles is induced by "panmixia." A more reasonable claim might be made out for the new genetic concept of "heterosis" as furnishing the function of sexuality and

[1] Paper read at the symposium on "The Biology of Sex" before the American Society of Naturalists, New Orleans, December 31, 1931.

sex. By heterosis we mean the increased vigor of hybrids, as compared with pure breeds, which is caused by the preponderant dominance of the genes favoring survival and growth furnished by both parents. But a more searching study of this matter shows that, in the main, heterosis affords only a compensatory advantage, in that it makes up for deficiencies that sexual reproduction is itself mostly to blame for. Heterosis arises only when cross breeding is wider than it has been on the average in previous generations. But if this wider cross-breeding is kept up, deleterious recessive genes will accumulate until a new equilibrium is reached, at which stage there is a sufficient abundance of such genes to cause even these more "mixed-blooded" individuals to exhibit as many recessive defects as did their "purer blooded" ancestors. *Vice versa,* if we increase the intensity of inbreeding, the more rigorous selection ensuing will eventually lead to the inbred line being purged till it has as great vigor as its more cross-bred ancestors. The closer the inbreeding, the less does sexual reproduction depart, in its genetic effects, from asexual reproduction, and we may conclude that at the limiting state, that of asexual reproduction, there would not (after the attainment of a state of equilibrium) be less vigor than in sexual organisms. The attainment of equilibrium in regard to the number of harmful mutant genes present may, however, require a very considerable time, and in the meantime sexual reproduction would be of advantage through its induction of heterosis. Heterosis may therefore have been of immediate value, in the first origination of sexuality, and so it may explain how sexuality happened to become established in the beginning, as Altenburg has suggested in an as yet unpublished work. But heterosis can not explain the major function of sexuality and why it has persisted in the long run, and acquired such complicated accessories.

Among the primary and accessory features of sexuality there must be considered not only the differentiation of

male from female germ cells, the differentiation of male from female sex organs, the separation of the sexes, with its associated mechanism of sex determination, and the differentiation of secondary sexual and "sex-limited" characters in general, but also the mechanism of Mendelian heredity itself, involving segregation of homologous chromosomes, independent assortment of non-homologous chromosomes and crossing over. Without sexual reproduction, the latter mechanisms are not called for, and would not continue to operate. Which, however, among the attributes mentioned, occupy a more primary and which a more secondary status? It is clear that not only is sexual reproduction necessary for the operation of segregation and recombination of chromosomes and chromosome parts, but, conversely, the latter are necessary in order that sexual reproduction may have any permanent value, while all the other characteristics of sexuality, though enhancing, are dispensable. Of the two major features, segregation and recombination,[2] only recombination is in itself of evolutionary value, but it can not take place without segregation and so we must suppose the two to have sprung into existence at nearly the same time. Mendelian heredity must therefore have arisen almost full fledged, when sexuality arose. This complicated step, which probably required a peculiar concatenation of accidents, along with selection, seems to have been taken in the green algae, and from them to have been inherited by animals and higher plants alike.

The essence of sexuality, then, is Mendelian recombination. Not increased variation in the sense of more change in the hereditary units or genes, now that we know there are these units, but the making and the testing out of all sorts of combinations among these gene mutations which would arise and become evident any way. Sexual-

[2] It is not possible at present to decide definitely whether recombination of whole chromosomes or crossing over was first evolved; either would have been sufficient to give value to sexuality. But it seems more probable that crossing over was a later development.

ity, through recombination, is a means for making the fullest use of the possibilities of gene mutations; thus it is itself an accessory process, accessory to the primary process of gene mutation.

There are two ways in which recombination of gene mutations is valuable. One, by far the lesser way, is the providing of an opportunity for continual shifting and readjustment of the relative abundance of different types as external conditions vary back and forth, and here and now one, there and then another combination becomes more advantageous for the maintenance of the species. In this process heterozygosity is an asset, and the disadvantageous combinations continually produced are an insurance against the day when some of them will be needed.

The other, the major value of recombination, is the production, among many misfits, of some combinations that are of permanent advantage to the species and that eventually become fully established in it as a part of its normal constitution. Without sexual reproduction, the various favorable mutations that occur must simply compete with each other, and either divide the field among themselves or crowd each other out till but the best adapted for the given conditions remains. In asexual organisms, before the descendants can acquire a combination of beneficial mutations, these must first have occurred in succession, within the same lines of descent. In sexual organisms, however, most of the beneficial mutations that occur simultaneously, or in different original lines of descent, can increase largely independently of one another and diffuse *through* one another, as it were (see Diagram 1). (Our diagram does not accurately represent this spread of genes through one another; it would hold only if the individuals and genes were fixed in geographical position and unable to disseminate freely amongst one another. If their positions were completely random, we should need a new dimension, at right angles to the previous ones, to represent the diffusion of each

new mutation. The actual situation lies somewhere between these two extreme alternatives.)

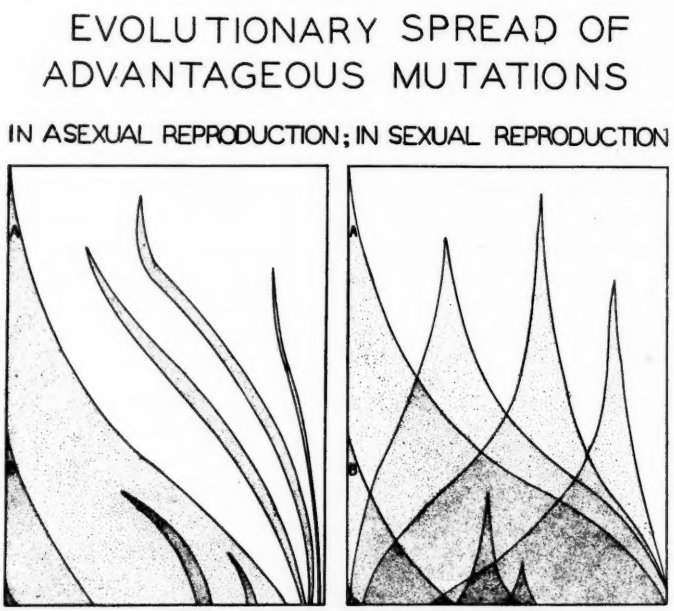

DIAGRAM 1. Showing the method of spreading of advantageous mutations in asexual and sexual organisms, respectively. Time is here the vertical dimension, progressing downwards. In the horizontal dimension a given population, stationary in total numbers, is represented. Sections of the population bearing advantageous mutant genes are darkened, proportionally to the number of such genes. In asexual organisms these genes compete and hinder one another's spread; in sexual organisms they spread through one another. See, however, qualifications in text (p. 121), explaining limitations of a diagram in only two dimensions. The diagram is simplified in a number of other ways as well. For example, all mutants represented are shown as spreading at nearly the same rate, if they do spread, and this rate is shown as about the same regardless of the extent to which they have entered into combination with one another.

Now it can easily be shown that the ratio which (on the average) the number of individuals in the most favored line of descent, counting from the time of occurrence of one favorable mutation (A) to the time of occurrence, within the same line, of the next favorable mutation (B),

bears to the number of individuals in the population as a whole in the same period (in Diagram 1, the ratio of the left-hand mutant shaded area A to the total area, in the region between two horizontal lines drawn through the points of origination of mutant shaded areas A and B) would represent roughly the speed of evolution in an asexual as compared with a sexual organism,[3] provided a correction, making the situation still more favorable to the sexual organism, is made here, namely, multiplication of this ratio by a factor representing the greater speed of increase of the favorable mutations in the sexual than in the asexual organisms, due to the fact that in the former the different favored mutations do not have nearly so much tendency to interfere with one another's increase. When such calculations are made, using any reasonable-seeming premises for mutation rate, selection and population size, within very wide limits, it is found that the advantage of sexual over asexual organisms in the evolutionary race is enormous.

In these calculations, and in the diagram, the assumption has been made, for the sake of simplicity, that the advantage of a mutation is the same regardless of the combination in which it occurs. However, the value of a combination of mutations will sometimes be far greater than the mere sum, or even the product, of the values of each mutant condition taken separately. Therefore, as Wright has recently pointed out, it is sometimes possible, by means of recombination occurring before selection, to get valuable combination-types which would not have come into existence at all, or only with far greater difficulty, if the "complementary" mutations composing them had had to occur and then to become selected in succession, as must happen in asexual reproduction.

[3] For while, in the given time, only one new advantageous mutation (B) became available in the favored line (A) of the asexual organism (the mutation rate being such as to give one in this number of individuals), in the sexual organism as many new advantageous mutations would become available, for combination with A, as the area of A goes into the total area.

While it is true that only the findings of modern genetics could enable our conception of the function of sexuality to take on the definite form above outlined, and only they could furnish real proof of this conception, nevertheless it should be recognized that the core of the idea—the formation of new combinations of "determinants," having a selective value sometimes greater than the original combinations—was conjured up long ago by the genius of Weismann, who herein, as in a number of his other major contentions (non-inheritance of acquired characters, reduction division), to-day stands brilliantly confirmed.[4]

II. On the Origin of Sex and Sex Determination

The advantage of the division of labor between sperm and eggs has long been obvious to biologists. Perhaps it is also needless to point out that the further differentiation, leading to the existence of the two sexes in separate individuals, is of advantage in the same way as any other division of labor in which more individuals than one are mutually involved, in this case rendering the performance of the respective tasks of finding a mate (or causing the male gametes to reach the female), and of giving the offspring a good start in life, respectively more efficient. But in cases where conditions are such that these functions in the same individual would not greatly interfere with one another—as is often true in organisms that are slow-moving any way and that need not be otherwise even for mate-finding—the efficiency may not be increased enough by dioeciousness to compensate for the effect of the latter in halving the number of individuals giving each type of gamete and in reducing the proportion of contacts which would be of service in fertilization, and so these organisms may have retained or developed hermaphroditism. This relation too has been pointed out by Altenburg in the work above referred to. His con-

[4] I am indebted to Professor S. J. Holmes for having redirected my attention to this important historical fact, subsequently to my address at New Orleans.

tribution concerning the relation of "reproductive load" to hermaphroditism is especially valuable in this connection, but we do not wish to anticipate it here. On the other hand, in dioecious species, it is not so evident why it should be most advantageous for them to have almost exactly equal numbers of the two sexes, as is usually the case, but perhaps this proportion exists simply because it is the easiest to produce, genetically.

If, as seems likely, hermaphroditism was the more primitive condition, dioeciousness may sometimes have arisen, as recently obtained artificially in corn, by means of two separate mutations which caused male-sterility and female-sterility, respectively. These mutations may in some cases have been linked (lying in homologous chromosomes), but such an arrangement would not be in the direction of the more prevalent mechanisms of sex-determination, in which the Y or W chromosome is relatively unimportant. More likely the second mutation (say, that causing female-sterility) was of a "sex-limited" type, such that its effect could be produced only when the effect of the first mutant gene in question (say, that causing male-sterility) was not being produced. Thus the second mutant gene would tend to become homozygous throughout the population and yet the effects of the two mutant genes would remain alternative. The first mutant would come to be heterozygous in half of the individuals and homozygous in the rest; it would have to be regarded as the "sex-determiner" proper. But it would seem a long way even from this kind of dioeciousness to one in which the sexes are automatically almost completely alternative, as in some animals that have been studied, where the development of a set of characteristics of one sex, in any given part of the body at any given stage, whether owing mainly to genetic or environic influences, necessarily goes along with a corresponding inhibition of a whole set of characteristics of the other sex. Many adaptive "modifying" mutations would have had to become established by selection, to

make the process controlled by the sex-determiner so effective, in causing the development of the characters of each sex to occur to the exclusion of those of the other sex.

As shown first by the work of Goldschmidt on the gipsy moth, the same genetic configuration may lead to either full maleness or full femaleness, for a given part at a given stage, independently of other stages, the result at that point depending on the strength of a particular developmental influence or influences, and in special cases the strength of the influence may change gradually during development so as to cross the critical level separating the two sexes. As the crossing of this level seems to involve a cleanly alternative alteration in nearly all sexual characteristics at once, that are scheduled to undergo development at the stage in question, it seems likely that the determining influence in question is a single one, *i.e.*, that there is normally a single "focal" process of development, or a single kind of developmental material, that is sex-deciding. That the same is probably true in Drosophila may be deduced from the recent work on intersexes by Dobzhansky and Bridges, extending Goldschmidt's principles to this organism. Further evidence of the largely unitary character of this sex-deciding process is to be found in the fact that the mechanism of sex determination has time and again changed, and that when the change occurred it could scarcely have been by a series of small steps, as would have had to be the case if many independent processes had been involved.

It is reasonable to suppose that the present scheme of sex-determination in Drosophila, for instance, arose as the result of a mutation which affected the strength (*i.e.*, the intensity or concentration) of the process or substance in question. This mutation need not have represented the first origination of the dioecious condition or of the sex-deciding process in question. The pair of allelomorphs thereby established may merely have superseded another allelomorphic pair, or another set of alternative conditions, which previously had had the sex-

deciding rôle, just as must have happened when what I have designated (in "The Mechanism of Mendelian Heredity," 1915, p. 83) as the "WZ" method of sex determination of butterflies and of birds superseded the previous XY type more generally characteristic of insects and of vertebrates, respectively. We are not compelled to conclude that the new gene or pair of genes that had this deciding effect was the one that synthesized the "focal" substance, or that chiefly carried on the "focal" process in question. No doubt, as in the production of other characters, this substance or process too depended, and depends, on many genes, some more and some less important, some helping to determine its nature, others only influencing its "strength," or allowing it to exist (see Diagram 2). In the same way, whether or not a pistol shall be fired may depend upon various details of the nature of the mechanism, upon the powder or merely upon the pulling of the trigger. To which of these possible categories the newly deciding gene belonged can not now be ascertained. But this single mutation must by itself have been enough to allow the gene in question to become fully sex-determining, *i.e.,* to decide between a fully functional male and functional female, otherwise the mutant would not have been able to survive.

Altenburg (*op. cit.*) has pointed out that there is at first sight an apparent genetic difficulty encountered in accounting for the origination of the above mutation, inasmuch as the work on non-disjunction shows that the X is far more important than is the Y in sex determination and that therefore the male represents, in effect, a haploid condition of sex-deciding genes present in diploid in the female. We may account for such a situation in one of two ways. On one interpretation we take as our point of departure the genotype of a male or hermophroditic individual, not containing the present sex-determining gene or genes which I have designated as "S" ("Mech. Mend. Hered.," 1915, p. 78) which now exist in the X chromosome, but homozygous for an earlier allelo-

morph, which we may call "s," and which we must suppose to be indifferent in its effect on sex. By means of a "positive" mutation (*i.e.,* one different in its character from a loss, and similar to Hairy wing, Blond and Bar in Drosophila—see Muller, League and Offermann, 1931, *Anat. Rec.,* 5: 110), the indifferent gene "s" would have had to become changed to the sex-deciding gene "S," which had to be present in double dose (SS) before its effect of suppressing maleness (while allowing development of femaleness) could be produced. The indifferent allelomorph "s" in the "Y chromosome" remained equivalent to an "absence," so far as its effect on the sex characters was concerned, just as the normal allelomorphs of Hairy wing, Blond and Bar are equivalent in their effects on these characters to absences.

On the other interpretation, we start from the homozygous "SS" individual (female or hermaphrodite) as a base. We must now suppose that a mutation of S occurs which is similar in its effect to a loss, producing an indifferent gene, or an absence, "s." This lesser gene, or loss, s, may be said to dominate over S, in the sense that the one dose of S, in the combination Ss, has a different effect than the two doses, SS, this difference being sex-deciding. The peculiar feature of this situation is not that one dose has a different effect from two, but that the sex-determining mechanism should already have been so prepared in advance, as it were, that a mere loss was all that was necessary to produce the whole change-over. This becomes understandable, however, if we postulate in this case that the "focal" sex-deciding substance or process had been previously evolved, through a series of changes in other genes but that a different gene or other agent had hitherto been determining whether or not it should occur. Once having gone through this evolution, its strength would be influenceable by changes in various contributing factors. Of these, the sex gene "S" here in question was merely one.

In view of these considerations, we should also expect that in the future changes in still other genes might be able to exercise the deciding influence and so take over the function of sex determination, in which case, in all probability, other chromosomes would become sex-determining. Moreover, there is nothing in the mechanism projected which would, *a priori,* make it impossible for quite different kinds of influences, such as special external conditions, or the haploid-diploid difference, or the stimulus of fertilization, to take over the function of the pulling of the trigger of the already evolved sex-determining process. But it would be to the advantage of the organism if the sex-determining process were not easily influenced by ordinary environic differences, for then the sex ratio would be too easily upset, and so we should expect the process to have developed safeguards against being readily changed in such ways to the degree necessary to cause the change-over.

III. Singular or Plural "Sex Genes"?

The fact that originally there must have been just one gene that played the critical rôle in the sex-deciding process does not mean that, as time went on, and the X-chromosome became, by mutation pressure, increasingly differentiated from the Y, other genes did not finally come to be contained in the X which also, through the difference in the effect of their dosage in the one-X and two-X conditions, affected the process in question. Some of these may work in the direction of strengthening, others of weakening this process (when their dosage is increased), but the sum total of their activities, together with that of the original sex-determiner, must be about the same as the latter originally was by itself. So there is as much likelihood that the X-chromosome contains "minus" sex-genes—those working in the male direction—as "plus" ones, and if such exist there must be an intra-chromosomal balance between the two. Recent work of the author, League and Offermann (1931,

op. cit.; see also Muller, 1930, in *J. Gen.*, 23) indicates that in the X, owing no doubt to its peculiar history, the intra-chromosomal "genic balance" is peculiarly delicate and intricate, in regard to the determination of various traits, and sex should be no exception to this.

As I pointed out in 1928 (Muller and Altenburg, *Anat. Rec.*, 41: 100) breakage of the X-chromosome by x-rays will enable us to determine the approximate locus or loci of the present sex-deciding gene or genes in the X, along with the real map of other genes. Hence, if the sex genes are not too numerous, their number and their relative effects also can be determined in this way. My work on broken chromosomes had already showed at that time that not more than half of the X contained genes of consequence in this connection. Since then the work has progressed a good deal further, chiefly through genetic studies of Patterson on broken chromosomes in mosaic flies in which the normal half of the zygote allows the other half, having the broken chromosome, to live and have its sex ascertained. As I suggested in 1930 (Muller and Stone, *Anat. Rec.*, 47), the inviability of the individual having a large piece of the X broken or missing, which is our chief difficulty in such studies, should also be obviable, to some extent, by carrying out our studies on flies triploid for the autosomes, since here the genic disproportion would not be so great. Following this method, Dobzhansky and Schultz (*Proc. Nat. Acad.*, 1931) recently report finding that several parts of the X have some positive influence in sex determination. It is too early to make a digest of the whole matter, but we can at least say that some parts of the X of *Drosophila melanogaster,* including all genes to the right of forked, are practically without influence in sex-decision, other parts, in the extreme left end, are of little if any influence, and in the remainder the influences are at least unequal, and are being gradually traced down. For the further definite findings of importance along this line the reader may be referred to the very extensive work of Patterson

(abstracted, 1931, in *Anat. Rec.*, 51: 111), which he has summarized for us at the present meeting, in his address to the genetics sections.

In all the above discussion it has of course been taken for granted that it is not the absolute amount of the sex-deciding gene or genes which determine sex (still less the quantity of the X-chromosome material as a whole), but the amount relatively to that of other genes, with the products of which those of the sex-deciding genes react. There is no use detailing here the controversy into which my advocacy of this view brought me in the early days with other Drosophila workers, notably Sturtevant, who cited in opposition sex determination in Hymenoptera, which I maintained was controlled by a totally different type of trigger, but its final vindication came, as is well known, with Bridges' discovery of triploid females and intersexes in Drosophila and his more recent demonstration of haploid female tissue as well. You can not tell how sweet a cake is merely by knowing the amount of sugar used in making it, you must also know the amount of the other constituents and its total size: the same principle holds in sex determination. By the same token, too, we must conclude that the amounts of various specific autosomal genes are also important in sex-determination and, by means of breakage produced by radiation, we should eventually be able to trace down these genes also, and gain information concerning their rôles in the sex-deciding process.

We can not, in this short space, discuss or even pretend to outline the problems in the whole field of the genetics of sex, but it should not be forgotten that what I have called the "sex-deciding" process is but one process, and maybe a relatively small one, in the whole series of processes that interweave in the production of all the male and female primary and secondary sex characters and many so-called sex-limited characters (see Diagram 2). I have called it a "focal" process because, firstly, no doubt the reactions set up by various genes converge as

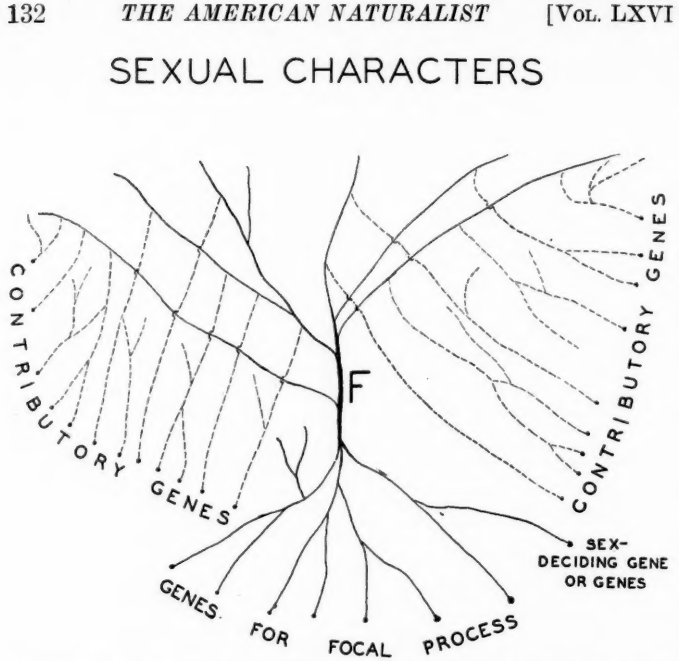

DIAGRAM 2. Schematic representation indicating the kinds of interrelationships existing between the final sex characters and the genes which determine them. Processes (physical and chemical, leading to morphogenetic effects) are indicated by lines, lines convergent from below upwards indicating interactions, lines divergent from below upwards indicating plural effects. The processes are represented as commencing with the genes below, and progressing upwards to the visible characters, above. Environic influences are not represented. Only one sex is shown. The "focal" or "sex-deciding" process, F, has the rôle not only of leading to the production of the characteristics of the one sex, but at the same time of inhibiting those of the other sex. This inhibition is not indicated here. Moreover, no distinction is made here between the direct production of a process and the "inhibition of an inhibition" of it, since the final effects are the same and a practical distinction can not usually be made. The diagram is admittedly vastly over-simplified, and is not intended to show the concrete relations of the processes, but only the kinds of relations existing among them.

to a focus, to make it what it is, to determine its nature and its intensity, and secondly, this process in its turn has multitudinous effects, lines of action diverging from it, as from the thither side of a focus, since the process

is necessary for the setting into action of all the various other processes which result in the production of all the different sex characteristics. But this multiplicity of effect also requires the cooperation of innumerable other genes, which, though not sex-deciding in the above sense, are certainly concerned with sex and are thus in a sense sex-genes. It may even be true that, to a slight extent, the majority of genes are influenced in the amount of their effects by the concentration of the sex-deciding genes, and are in this sense partially sex-genes, inasmuch as they tend to add to the sexual dimorphism.

IV. Rôle of the Y Chromosome

I have neglected the Y-chromosome in the above account, because the Y has neglected itself. According to the hypothesis of the origin of the Y which I published in 1914 (*J. Exp. Zool.*, 17: p. 326–328; see also *Gen.* 3, 1918, p. 479–484) the genes of the Y have gradually undergone inactivating and loss mutations, from the effects of which the organism has been largely protected, through the continual presence of an X having normal (or "hypermorphic") allelomorphs. In other words, the Y has paid the penalty always exacted by the protection of continual heterozygosis, and the consequent absence of natural selection. The largely inert Y may subsequently undergo changes in size and shape without detriment to the organism, and so tends to become visibly different from the X, luckily for cytologists and geneticists.[5] But it must

[5] Recent evidence (see Muller, League and Offermann, *op. cit.*) suggests that, although the individual "loss mutations" or "hypomorphic mutations" of the Y were not detrimental enough to prevent them from finally becoming established by mutation pressure throughout the population, nevertheless in the case of some of them, the ensuing one-dose condition of the genes of the X in the male may have exerted some sensible detrimental effect. If so, this effect was later compensated for by the selection of modifiers, which made the males with one dose about equal, in degree of expression of these X-chromosomal genes, to females with the two doses. If this interpretation is correct, loss of a whole section of the Y would have been detrimental, at a time before this piecemeal loss, accompanied by piecemeal compensation, had taken place. Alternatively, we may sup-

retain enough genes to allow it to act as the homologue of the X in segregation, if it is to persist at all, and, if any dominant genes exist or arise in it, which are advantageous to the sex in which Y occurs exclusively, they may be retained by natural selection. With regard to these male-helping genes we may observe the following: as it is a rule of evolution that characters at first merely an asset will, if they are retained long enough, finally become, through correlative evolutionary changes, a necessity, we find that at present the Y of Drosophila, though relatively unimportant in sex determination, nevertheless contains genes or gene-complexes, "k_1" and "k_2" found by Stern, 1927 (*Die Naturwiss.* Jahrg. 15) that are essential for the complete functioning of the male.

The Y, having become nearly inert, may also now serve as a source of inert chromatin that may become translocated into other positions, and even serve as an anchorage for the formation, by translocation, of new autosomes chipped off the old ones. At least, on the basis of the studies which have recently been carried on by Stone, Painter and myself, I have reached the conclusion that, by translocation, a large part of the Y of Drosophila has become engrafted on to the original X, so that only about half the length of the present X consists of original X-chromosome material, the rest consisting of inert material derived from the Y, material in the main unable to mutate, to undergo crossing over, or to function in morphogenesis (Muller and Painter, 1932, *Z. ind. Abst. u. Vererb.,* in press). Its function, if any, still remains a mystery, but the possibility of its existence in unsuspected situations must be taken into account in future cytological studies. Here we have one little example of the numerous ramifications of the secondary effects which sexual differentiation has led to.

pose that these "compensated" genes of the X are "neomorphs," functionally unlike any that ever existed in the Y. But even in that case, *duplications* of the Y or of whole sections of it could not have been innocuous until it had become nearly "empty."

V. Significance of Sex Studies

The genetic study of sex is important not merely because, according to Freud, sex is instinctively our major interest, a proposition which is at least disputable, even for *Homo sapiens biologensis.* It is important also because, as we have seen, it lies at the root of Mendelian heredity itself and is one of the major factors in evolution, even though it is not, in an absolute sense, necessary. Thirdly, it is important because it provides such admirable material for the study of gene interaction, of phaenogenetics, that is, of *Entwicklungsmechanik* from a genetic standpoint, together (fourthly) with the associated study of the evolutionary processes whereby these developmental complications arose. Sex and sex characters are peculiarly adapted for this purpose because, while they constitute a highly complicated mechanism developed through a long evolutionary sequence, nevertheless they can be dissected apart, practically down to their very root, by a combination of mutational, embryological and physiological means, without necessarily killing the organism (as witnessed by the results cited in the other papers in this symposium). This possibility arises from the fact that the vegetative functions can go on without reproduction and that the organism, through its possession of two sexes, must be already adapted to carry on either one without the other. Most other complicated systems—circulatory, digestive, excretory, etc.,—if much tampered with, soon result in lethal effects, and thus largely foil the knife of the genetic and morphogenetic investigator. Within the life system as a whole, then, we have here a contained system, the sex system, sufficiently independent so that it can be vivisected down to the point of extirpation without death ensuing, and which can therefore be used as an object of research to illustrate the general principles of gene interaction, morphogenesis, physiology and evolution pertaining to the life system as a whole.

APPENDIX

The reader may wonder why the letter "S" has been used in the preceding article to represent the sex-deciding gene or group of genes, rather than the letter "F," for femaleness, and why "M," for maleness, has been omitted completely. He may also think that some references should have been made to the development of these F- and M-containing formulae for sex, and to the controversy concerning to whom the credit for these conceptions should be given. In view of the prominence which has been given to this topic even in some of the more recent literature, the author ventures to present here a portion of a paper entitled "Erroneous Assumptions regarding Genes," which he wrote while at the Cornell Medical College in the winter of 1911–12, and which he did not have opportunity at that time to publish. It will be evident from this paper that even at that time the "FM" formulation was no new one, but that the grounds for its rejection were already extant. As a result of the ideas of the author advanced therein, and others added by him later, the representation "SS, SO" was used in the "Mechanism of Mendelian Heredity," 1915, 1923, and the criticism "What are Sex Factors," was presented in the same volume (see especially pp. 90–97 and p. 107 of the 1915 edition, or pp. 90–94 of the 1923 edition, which it may be of interest to reread in the present connection).

. . . We may in conclusion undertake a criticism of certain needless assumptions that are sometimes made regarding sex itself, for this may furnish an illustration of the applicability of the above line of thought (that the processes through which the genes accomplish their outward effects may be extremely complex and interrelated) in other directions. When we find that the male, for instance, is heterozygous for sex, the female homozygous, then, in the absence of any cytological evidence, there is only one justifiable formula by which the sexes can be expressed, namely XX = ♀, XY = ♂; or, if there is evidence from cytology that the X chromosome has no mate, the Y may be changed to O. All other formulae are unwarranted modifications of these two. Thus, Morgan, in an attempt to account for maleness in Drosophila (where the Y is absent and the scheme is XX = ♀, XO = ♂[6]),

[6] Just at that time, the Y was erroneously thought to be absent from Drosophila. Nevertheless, as it has since been found to be nearly "empty" genetically, this formulation will hold approximately anyway.

introduces an M into the formula and changes X to F. His formula for the ♀ is then FMFM, that for the ♂ FMM, where F, femaleness, stands for X, and M, maleness, is not sex-linked. Both of these assumptions are unwarranted. It may well be that not only factors necessary for femaleness but also factors for maleness abide with the X chromosomes. This may be true not only of sex characters common to both male and female . . . but even of distinctively male characters, or generalized "maleness" itself, if that exist. . . . For, just as a gene in haploid amount need not produce an effect equal in quantity to that of the diploid amount . . . , so too its effect may differ also qualitatively, when haploid, from the diploid effect. It is not uncommon for the effect produced by an agent to vary in quality as the agent varies in quantity. Thus, to build upon an idea of G. H. Shull's, a certain amount of alkali added to an acid solution yellow with alizarin, may change the latter to an orange color, whereas twice as much may make it deep purple. Therefore it is quite possible that XO = male, XX = female, without any non-sex-limited M, and therefore too that X is not identical with a hypothetical F, femaleness.

It is not even necessary, however, to postulate here that a certain gene in haploid number produces an effect different from that in diploid. We may instead assume that certain dominant factors for maleness exist "in" the X chromosome, but that there also exists in that chromosome a recessive inhibitor for this factor or factors (either a simple inhibitor or a complex of several genes that may have other side actions as well). We say recessive, meaning that the haploid quantity is ineffective. In this case the composition of the X chromosome, if represented according to the scheme of the preceding formula, would be MI; this too would represent the male, one I not being active, and M thus producing maleness. The female would be MIMI, the two I's effectually inhibiting maleness. In this case we could consider femaleness to be a non-sex-linked character or aggregate of characters, thus partially reversing Morgan's formula of FFMM = ♀, FMM = ♂ by putting MIMIFF = ♀ MIFF = ♂; we could, however, as well consider that "femaleness" (meaning all or a part of the factors necessary for femaleness) also was sex-linked, but recessive, like I, not dominant, like M. Then we would have MIF = ♂; MIF MIF = ♀. If we chose, we could simplify the formula by the omission of I, regarding F (or even M) as possessing its properties, especially since by the letters we are not strictly denoting single genes, but possibly collections of them. Then we would have MF = ♂ MFMF = ♀, M dominant to its absence, F recessive to its absence and inhibiting M. If likewise in the previous case (MIMIFF = ♀, MIFF = ♂) we considered I as being merely a property of M, we would have MMFF = ♀; MFF = ♂.

In all the above cases, we have for purposes of comparison used the scheme of representation of the formula under criticism; we do not favor this method of representation in general. For, as we pointed out in another connection, a single letter is used in Mendelian notation to represent a single gene, but, in cases where the factor has never been actually isolated . . . , we have no means of telling whether the effect is not due to the combined action of a number of genes, any of which may in their

turn be responsible for other effects as well. Thus, if when using F a single gene is meant, the assumption is unwarranted; if a possible collection is meant, the notation is unwarranted. In case F or any other such "character" is sex-linked, however, (as F is in Morgan's formula), this representation is somewhat more defensible, for in this particular case the character always segregates completely as a unit in the heterozygous sex, and the integrity of the letter is destroyed only by mutation. It seems therefore permissible to use a single letter here, provided we call attention to the exceptional nature of the case. . . .

However, if the "character" may be made up of a collection of non-sex-linked genes, it should never be represented in this way, for then the various components need not all be coupled together as they are in sex-linked cases. The integrity of the letter is thus destroyable by their independent segregation, whereas the use of a single letter implies that in segregation its genic original acts as a unit. This objection applies to the M of the formula under discussion, even if we regard the formula as correct in intention.

To pursue the case to its conclusion, objection is to be raised to the insertion of MM into the formula also because it is provided in diploid quantity throughout (♀ = FFMM, ♂ = FMM). The representation of any factor or factors which appear in like quantity throughout any complete series of cases, is superfluous. It is not legitimate to answer to this that the gene does not manifest itself in all these cases. Thus, in the case of the fly with the abnormal abdomen, let us assume, as is usual, that abnormality, the dominant, is also the presence. Then the abnormal fly is represented by AA or Aa, and the normal by aa. Normal is represented as the absence of abnormality, and yet for the production of the normal abdomen many factors, which together might be called NN, normality, are surely necessary. However, as these factors are present in all cases, they are not represented, even though they are in evidence as such only when A is absent and work differently or not at all in its presence (*i.e.*, do not manifest themselves), just as is the case with the hypothetical MM. If a certain somatic effect is produced, its representation in the germ cells is presupposed, and essential likeness of genic composition is assumed except where difference is indicated in the formula.

We have laid stress upon method of notation because a clear agreement as to method of symbolization is important for clarity of thought regarding the objects of the symbols. And as regards these objects, the genes, we hope we have indicated that, in regard to presence and absence and some other questions, it is necessary to conjecture freely and fully as to all the possibilities involved, if one wishes to avoid the paradoxical criticism—consequent upon the missteps which come inevitably to those who try to reach "natural" conclusions but avoid "unbridled theorizing"—that one has actually indulged in wild speculations. . . .

THE AMERICAN SOCIETY OF ZOOLOGISTS

CHROMOSOME MOVEMENTS[1]

PROFESSOR C. E. McCLUNG
UNIVERSITY OF PENNSYLVANIA

THE substance of living things is unique, and marked by a peculiarity entirely lacking in the non-living—the power to reproduce itself in kind. In the operation of this process the unit of structure—the cell—is also the unit of function. The general details of cell reproduction are now well known and the importance of certain small elements, the chromosomes, fully recognized. These small bodies, approaching sometimes the limit of visibility, give every indication of being primarily responsible for the process of reproduction, because, before the cell can divide into two cells, each chromosome must accurately duplicate itself. This process is one of incalculable nicety and precision—the minute and definitely arranged subdivisions of almost molecular size each reproducing itself with greatest accuracy. The chromosomes and their parts have been found to correspond in their behavior most precisely with that of the characters represented in parents and offspring, so that biologists can many times predict the behavior of parental characters in their progeny. No element of structure or detail of movement is therefore without significance. A study of the chromosomes during their reproduction reveals many complicated movements, some of which suggest ready explanations, others being, as yet, without reasonable explanation. A plea is made that a more intensive study be undertaken of chromosome movements in the hope that a better understanding of them may lead to some explanation of the unknown processes of development and differentiation.

[1] Paper read by invitation before the New Orleans meeting of the American Society of Zoologists, December, 1931.

Why should time be given to the consideration of such a topic as this? Are not the chromosomes divided in the metaphase of mitosis and pulled to the poles of the spindle in the anaphase—and is not this the extent of their movements? A casual inspection of the literature might justify such questions, since rarely is there a full and critical discussion of the history of chromosome behavior. So long has attention been focused upon the striking appearance of the chromosomes in the mitotic figure that one almost inevitably visualizes them thus when they are mentioned. A tacit assumption that they are so limited appears in many discussions where the chromosomes are said to make their appearance by concentration out of a reticulum or even by some physical process of crystallization. There is occasion, it seems to me, to consider with care the manifold and exquisitely ordered transformations which manifest themselves with such bewildering detail in most cells.

There is something to excite wonder and admiration in the nicety and precision with which, on so small a stage, these little actors in life's drama play their so significant parts. Not only in their relatively larger manifestations are they interesting, but even in those details which approach the ultra-microscopic they intrigue us. The endless and innumerable repetitions of the same purposeful chromosomal program in organisms of high and low degree indicate most clearly the profound significance attaching to each detail and challenge our critical attention. Here, perhaps, lies concealed an explanation of those perennial biological problems of reproduction and differentiation. So much has been done to explain the relations of generations to each other by consulting the behavior of their germ cells that we are led to hope we may likewise decipher the cryptogram of their development. There must certainly be significance in each twist and turn of these fateful threads. They offer in their weavings in and out and in their comings and goings through the intricate dance of mitosis a basis

for attack upon that still unsolved mystery of differentiation in development. What is there, in a process obviously and perfectly designed to secure exact duplication of every minutest detail of the determining chromatin during mitosis, that will insure diversity in the resulting sister cells? This is indeed a paradox, but only so because we have failed to grasp the significance of some less obvious phenomena. If the chromatin is, as we have increasing reason to believe, the governing element in the cellular complex, then certainly a better understanding of all its changes must contribute to a solution of the problem of differentiation.

I regret that I do not bring you solutions, but only suggestions. It would be so satisfying if I could point out even some promising lines of approach to an understanding of the methods of differentiation in cells, but so far we wander in darkness. The most I can hope to do is to emphasize again the need for a reattack upon this basic problem. In its solution lies the hope of a much more complete understanding of all life phenomena, normal and pathological.

If we hope to get inspiration and help from what knowledge we have, the first step would seem to be the familiar one of analyzing and ordering the facts which we already possess. Accordingly, I will ask you to consider with me such information as we have regarding the form, extent and character of chromosome movements, because certainly within them will be found the key to our understanding of their significance.

It is so necessary, in the beginning, to conceive properly the physical characteristics of the chromosomes, the nature of their substance and the size of its aggregates, and the proportion of the parts, if we are to understand them. Until recent years brought a restudy of the living cell, general conceptions of the physical characteristics of the chromosomes were colored by the images seen in fixed material. It was difficult to imagine the solid threads of chromatin, thus revealed, as active, moving

bodies, although it was perfectly evident from a succession of stages that they must move. Actual observation of their activities and an experimental insight into their physical characteristics through microdissection, make appreciation of their real nature easier. There is now no difficulty in understanding the possibility of all the varied changes of which the highly labile chromatin gives ample evidence. But to explain why and how these manifestations occur is quite another matter. It can not be without significance that cells themselves are very small bodies and even in the largest of them the chromosomes are very minute objects.

Their subdivisions, or chromomeres, are near the limit of the visible, and must be approaching the molecular in size. These are the bodies for whose movements we seek explanation. They are so small that in a dead cell they would exhibit Brownian movement, but in the living condition, instead of this purposeless vibration, they show ordered and predictable changes. Because of the fact that our analysis of visible structures demonstrates that, even so far as we can see discrete elements, they are showing purposeful movements, we must reasonably conclude that they are continued to whatever final organized bodies there may be—let us say the molecules.

Ultimately, of course, whatever activities there are in the chromosomes, they must be molecular, but certainly these differ in some essential way from those of non-living matter—we say they differ essentially because the results are unique. If reproduction is the quality of living things which marks them most fundamentally in contrast to the non-living, and if chromatin is the substance which governs the activities of the vital material, then there is no escape from the conclusion that it must inaugurate the process. The conspicuous part it plays in the cells set apart for the preservation of the species strongly confirms this.

What the initial act may be in this apparently teleological behavior is beyond the reach of our direct obser-

vation, but an understanding of it is the goal of all biological investigation. In the absence of any positive information, we must make assumptions to serve as guides in the search for fuller knowledge. It is an exercise of the imagination which can do no harm and may lead to some comprehension of this profound mystery. The more we learn about the process of cell reproduction by mitosis the more apparent it seems that the chromatin can not long exist in a thread of a single series of chromomeres. Reports of splitting or duplication of the chromosomes in the telophase are frequent; of this act in the anaphase, occasional; and of its occurrence in the metaphase of the mitosis preceding separation in metakinesis rare, but apparently authentic. If such a condition were common, then it would seem that it is impossible for the chromatin units to exist in an unpaired state. Should this be the case it would indicate that the characteristic molecules must primarily be incapable of an unbalanced existence. This would do no violence to our knowledge of organic molecules in general, for valence must be satisfied. It would be necessary to assume only that the chromatin molecule had reached such a development that it must have, not only polarity, but also a bilateral symmetry.

There remains, of course, the crucial question of why, having attained such a balanced state, the chromatin molecule should destroy it through division. Various possibilities suggest themselves in answer. Thus, for instance, it might be that the number of atoms, electrons, negative charges, or whatever it is that constitutes molecules, can not exceed a certain number without breaking the bond with the proton, or other central element, of the system. A reversal of electrical polarity in certain elements might produce repulsion where attraction previously existed. Changes in other elements of the cell system might bring about division of the chromatin units, although in view of the apparently causal nature of the chromosomes in the cell, it is much more reason-

able to assume that reproductive activities originate within them.

Whatever the primary source of this reproductive activity may be, the visible effect is a movement of repulsion, separation or splitting of such a nature that the two resulting elements are practically identical. One is tempted to inquire whether the appearance of a mirror image relationship reflects an inner organization of this type. If so, it might be easier to understand the tendency or, indeed, the necessity, of the chromatin always to exist in a double thread state. We are so accustomed to a bilateral symmetry in animals that it would satisfy an intellectual conformism to find it existing in their ultimate control units. Polarity, so characteristic of organisms, certainly exists, and a bilateral arrangement is obvious, but whether this extends to the constituent molecules is, of course, beyond our observation.

An interpretation of the movements of chromosomes must be, as in all biological matters, in terms of cause and effect. Why are all these intricate and involved processes necessary? They seem to be principally concerned with only two of the general functions of protoplasm—metabolism and reproduction, and, in a final analysis, probably in the main with the latter. We must therefore seek for explanations of how chromosome movements facilitate reproduction. All our experimental observations seem to indicate that the substance of chromosomes is the governing agent in reproduction. We also note that, in any reproductive act, the chromatin is the first material involved. Before a cell can divide its chromosomes must be duplicated. As Roux long ago observed, the essential act here is the accurate duplication of the finest observable subdivision of the chromatin thread—the chromomere. Linear extension obviously provides the most favorable physical conditions for this almost molecular duplication of parts. The reason for such an act is now much more apparent in the concordance with genetical results than it was when Roux gave

his explanation for precision of mitotic division in respect to the chromatin. One form of the movement of chromosomes receives, therefore, a ready explanation in their reproductive act.

If the stretching out of the chromatin thread facilitates the subdivisions of its finer elements, so also does its contraction into the dense metaphase elements make easier the translocation of the chromatid to the poles of the spindle. The opposite acts of extension and contraction are of obvious mechanical advantage when their purposes are considered. Since they are of universal occurrence in plant and animal mitoses, they result from the common necessities of all cells.

Other types of chromosome behavior do not find so ready explanation. Why should it be necessary for the elongated chromosomes to "act like eels in a basket," as Strangeways describes them from the study of living cells.

In many spermatocytes there is a definite looping of the chromosomes in the peritene stage, but in those of other species this is lacking. Such behavior can not therefore be of the general significance which marks linear extension and contraction in all mitotic chromosomes. Even so it characterizes the spermatocytes of perhaps a majority of animal species and must represent some important function. Previous to this sweep about the nucleus in the peritene stage, the chromosomes have been arranged in the diatene with their ends attached at opposite poles of the nucleus, but in making the loop, one end detaches itself and comes to lie beside the opposite end—producing the so-called bouquet stage. A new form of polarity is thus set up, but the reason for it is not obvious.

From all the available facts it seems reasonable to conclude that the chromosomes are definite, discrete bodies, capable of automatic movements of a highly diversified character. These movements must be directly related, causally, to the activities of the cell, particularly to its

reproductive function. Pursued to a logical conclusion, this must mean that the ultimate units of structure, molecules if they be such, reproduce themselves as the primary step of a series of duplications which finally involve the cell as a whole. Many of the complicated changes of the chromosomes suggest at present no causal significance, but it is unreasonable to suppose that this is lacking. The difficulty is that we have as yet no clue to guide us in our search for the meaning.

Ultra-violet photographs of living cells resemble in almost every respect the pictures of fixed and stained preparations. The validity of the images we have so long studied is now beyond question. We may undertake the study of the intricate maneuvers of the chromosomes, secure in the belief that what we see in the succession of static pictures represents truly the continuous process of the living cell. Here is a problem of utmost theoretical significance, one to challenge the best of technical effort, accuracy of observation, and, above all, of constructive imagination. The deceptive ease of experimental method has attracted many to its use in the hope that thus might be reached a knowledge of the rôle of the chromosome in cell processes. Much as these new disciplines have added to our knowledge, they are no substitute for that painstaking, laborious, and cumulative method of observation and inference which has already given us most of what we know of the chromosomes. There is much need for more capable workers in the study of the eternal dance of the chromosomes in reproduction, and I hope that an appreciation of the possibilities here may lead to many accessions to the ranks of the observational cytologists.

ON THE TROPHIC IMPULSE SO-CALLED, ITS RATE AND NATURE[1]

PROFESSOR G. H. PARKER
ZOOLOGICAL LABORATORIES, HARVARD UNIVERSITY

THE general subject of trophic impulses as well as of trophic nerves has long been one of uncertainty and obscurity. To the neurologist of a century ago nerves were either sensory or motor, but this simple classification was not to last long. In 1845 the Webers discovered, somewhat accidentally, that the stimulation of the vagus nerve did not induce the beat of the heart, but, on the contrary, checked the action of that organ. Thus ground was cleared for the idea of inhibitory nerves. Six years later, in 1851, Ludwig in his study of the salivary glands of the dog advanced strong evidence in favor of secretory nerves. In this way by gradual steps new categories of nerves were added to the original two.

As early as 1823 Mayo called attention to the fact that after cutting the trigeminal nerve the conjunctiva of the eye became inflamed, the cornea ulcerated and the face on the operated side edematous. These conditions were subsequently produced experimentally in lower animals by Toderà, Magendie and Longet. Although a number of workers openly disagreed with these results others confirmed them, and this confirmatory evidence together with much more of a generally similar character led several of these early investigators to conclude that beside the kinds of nerve-fibers already enumerated, trophic fibers must be added to the list. These fibers were believed to spread through the nerves of the body in association with sensory, motor and autonomic elements, and to be profoundly important in determining the metabolic activities of the tissues to which they were

[1] Paper read by invitation before the New Orleans meeting of the American Society of Zoologists, December, 1931.

distributed. This idea was clearly and forcibly expressed by Samuel, who, in a volume entitled "Die trophischen Nerven" published in 1860, declared that "der Grund der Ernährung liegt in den Zellen, das Mass der Ernährung in den trophischen Nerven." Thus Samuel may be regarded as the founder of the trophic-nerve theory, a theory that met with a favorable reception from such distinguished investigators as Weir Mitchell ('72) among the older workers and L. R. Müller ('24) among the moderns.

But it must not be thought that the theory of trophic nerves was by any means generally accepted. Virchow ('68), with his strong predilection for the independence of the cell, opposed it vigorously, and in this he was folfowed by Cohnheim ('82) among the earlier investigators and more recently by Roux ('10), Mönckeberg ('15) and Ricker ('24). No one could deny after the strong confirmatory evidence brought forward by Claude Bernard ('68) that the cutting of the trigeminal nerve was ordinarily followed by serious consequence to the eye, but it was believed by most students of the subject that these consequences were due to other causes than the loss of trophic fibers. Both Snellen and Donders showed that after severing the trigeminal the inflammation of the eye could be postponed six to ten days if care was taken to protect that organ against mechanical injury and the irritation due to dust. Thus it appeared that the resultant panophthalmitis was not due to the loss of trophic fibers so much as to the loss of sensibility, a loss which prevented the ordinary protective and cleansing reflexes of the eye. Such an explanation, however, could not be applied to muscles which, as is well known, atrophy after the nerves that supply them have been cut. This degeneration, however, was commonly ascribed to loss of normal function, in brief to inactivity, a state which is usually associated with deficient circulation. As a matter of fact, a considerable number of investigators ascribed all trophic disturbances to vasomotor maladjustments

resulting from nerve cutting. Such workers admitted the existence of trophic influences, but like many others saw no reason for assuming specific trophic fibers.

From the mass of conflicting evidence that this general problem has called forth and that has recently been very fully surveyed by Fleischhacker ('27), it is impossible to draw any safe conclusion. The most that can be done at the present juncture is to state in a rather categorical manner the various explanations that have been offered from time to time for those obvious conditions that have been grouped under the general head of trophic disturbances. These disturbances, according to the numerous investigators who have interested themselves in this field of research, have been assumed to be produced in one or more of the following ways: (1) By interference with the normal activity of true trophic nerve-fibers; (2) by interference with intercellular metabolic relations in which nerves are no more important than other types of tissue; (3) by interference with the local blood-supply usually through vasomotor irregularities; (4) by interference with peripheral sensibility whereby the normal protective reflexes are prevented; (5) by interference with normal functional activity resulting in inaction. The instance of trophic activity to be considered in this paper will be discussed from the standpoint of these five assumed explanations.

The lateral-line nerve of the common catfish, *Ameiurus nebulosus* (Les.), is a very convenient nerve for the study of trophic activities. It extends as a subcutaneous bundle of fibers from the rear of the head of the fish posteriorly over its flank nearly to its tail. In its passage from the head it diminishes in size by giving off small branches to two dozen or more lateral-line organs located in the lateral-line canal which extends, parallel with the nerve, along the side of the fish. This canal is immediately under the skin and opens freely to the exterior by pores which mark on the surface of the animal the so-called lateral line. The lateral-line organs are large

sense-buds located segmentally in the epithelial lining of the canal. As previous work has shown (Parker and Van Heusen, '17), they are receptors probably stimulated by movements of the surrounding water, including vibrations of low frequency.

For experimental purposes the lateral-line nerve is conveniently accessible immediately under the skin of the catfish in the region just below its dorsal fin. Here the nerve may be readily cut, after which the slight incision necessary for the operation quickly heals and the fish recovers. Brockelbank ('22, '25) has studied the effect on the lateral-line sense-buds of cutting this nerve. The buds anterior to the cut, that is on the proximal segment of the nerve, remain unaffected; those posterior to it gradually degenerate. On the fourth day after the operation, only slight traces of beginning disintegration can be seen in them. Their degeneration, however, may be complete in some instances in as early as seven days, in others not until thirty-five or more days. If the fish is allowed to live for some time after the operation, regeneration of the sense-buds invariably sets in. This may be well advanced, according to Brockelbank's observations, at fifty-four days after the cutting of the nerve and may be practically complete at a hundred and sixteen days. In these respects the lateral-line buds show a general agreement with taste-buds. Presumably as in the latter, the degeneration and regeneration of the sense-buds are dependent upon corresponding changes in the associated nerve; in other words, the nerve exhibits a profound trophic influence over the buds in that these receptors disappear with the degeneration of the nerve and reappear with its regeneration.

From the tables illustrating Brockelbank's paper, it is clear that the lateral-line buds do not degenerate and regenerate all at one time. There is some indication that in these operations a certain amount of sequence is followed. As shown in Table 1, the first bud posterior to the cut in different fishes was only partly degenerated at

4, 7 and 10 days, whereas, excepting at 21 days, it was completely degenerated at 13, 16 and 35 days. The second bud was, excepting at 7 days, only partly degenerated at 4 and 10 days, but completely so at 13, 16, 21 and 35 days. Thus degeneration must be assumed to be a relatively slow process in the catfish arriving at its completion only after some dozen days or so. If now the table is inspected for the sequence of degeneration, a certain amount of favorable evidence is to be seen. Thus at the four-day stage the ten buds which constitute the series that extended from anterior to posterior over the lateral line in a given fish were all in the condition of only partial degeneration. At the thirty-five-day stage the anterior part of the corresponding series is composed of nine completely degenerated buds followed by three partly degenerated buds and one fully degenerated. These conditions suggest that after the nerve is cut a wave of incomplete degeneration passes over the lateral-line organs marking the initial stages of this process and followed later by one of complete degeneration. It must be confessed that the rest of the table shows very little evidence either for or against this hypothesis, but the first and last of the records in this series are distinctly favorable to such a conclusion.

The degeneration of the lateral-line organs presumably follows the degeneration of the lateral-line nerve and if these organs degenerate in succession and not simultaneously, it is reasonable to expect that the nerve has done the same. The effect of cutting the lateral-line nerve on the severed part of the nerve itself has, therefore, been studied by Mrs. V. L. Paine and myself with the view of ascertaining the character of its degeneration. Does the lateral-line nerve after separation from its central connections degenerate progressively as the series of buds seems to indicate or does it disintegrate simultaneously throughout its length? The current opinion among the majority of students of nerve degeneration is to the effect that nerves, after severance from

their trophic centers, degenerate throughout their extent simultaneously. Of recent workers Bethe ('03) is the principal one who has opposed this opinion and who has advocated progressive degeneration in place of a simultaneous process.

To test this question on the catfish, we cut the lateral-line nerve on one side and studied its degeneration as indicated by the disintegration of its medullary sheath. When degeneration first set in it was impossible to distinguish any difference between the conditions of the nerve-fibers near the cut and at some five centimeters or so posterior to it. A comparison of these regions would have led to the conclusion that degeneration was simultaneous. When, however, two such regions were compared twelve to thirteen days after the operation, an unmistakable difference could be observed and it was clear that at that stage the region of the nerve near the cut was farther advanced in degeneration than that far from this point. This general condition was repeatedly observed in numerous preparations and was so manifestly present that we have no hesitation in concluding that the lateral-line nerve of this fish after some twelve days of separation from its trophic centers shows unmistakable evidence of progressive degeneration and that this degeneration spreads from the center toward the periphery. In this conclusion we agree completely with Bethe and are opposed to those workers on nerve degeneration, who claim that this process is strictly simultaneous.

The conclusion arrived at in the last paragraph is entirely consistent with what little is known of the degeneration of the lateral-line sense-buds. Both nerve and buds appear to degenerate progressively in that after cutting the nerve the anterior or proximal part of the system degenerates in advance of the posterior portion. It is interesting to note that the evidence for this state is drawn independently from the condition of the nerve and that of the buds. Furthermore, the evidence of its occurrence presents itself in the sense-buds and in the nerve

at almost precisely the same time, namely, at about twelve to thirteen days after the cutting of the nerve. At this stage the degeneration of the nerve and of the buds is in full progress.

The regeneration of the lateral-line sense-buds, as shown by Brockelbank's studies, is clearly a progressive one. This is well shown in Table 2 of her paper. But this process, which takes place between about the forty-fifth and sixty-fifth day after the operation, is not one which involves a process closely comparable with the degenerative one already discussed. It is the result of the outgrowth of new fibers from the central cut end of the nerve, an obviously progressive operation. This growth of new nerve substance is of course gradual and spreads peripherally (posteriorly). It is, therefore, not surprising that the regeneration of the sense-buds should take a corresponding course.

If the degeneration of the lateral-line nerve is a process that progresses over the nerve from the cut end peripherally instead of one that affects the whole length of the nerve simultaneously, it ought to be possible to obtain some measure of the rate of this progress. The idea of such a progressive change is doubtless what has been in the minds of those authors who in previous discussions of this subject have used the expression trophic impulse or metabolic impulse, both of which imply some relation to the nerve impulse or chain of events exhibited by a nerve in its normal activity of transmission. No one, so far as we are aware, has ever attempted to measure the rate of the nerve impulse on the lateral-line nerve of the catfish, but judging from measurements of such rates on the nerves of other cold-blooded vertebrates, it might be expected to be in the catfish not far from twenty-five meters per second. Is there any reason to suppose that rates of this order of magnitude occur in the passage of the so-called trophic impulses over such a nerve as that of the lateral line? For a test of this kind the lateral-line nerve is most satisfactory, for its func-

tional relations are such that the two kinds of influences that pass over it do so in opposite directions. Thus the normal sensory impulses transmitted by this nerve arise in the lateral-line sense-organs and travel thence anteriorly over the nerve to enter the brain in the region of the medulla oblongata. The trophic or metabolic influences on the other hand, as shown in this paper, progress from the central region of the nerve, presumably from its ganglion-cell-bodies, peripherally to the sense-buds. Thus the sensory impulses take a centripetal course and the trophic influences a centrifugal one. Hence the favorableness of this material for the measurement of the rate of the assumed trophic impulses.

To ascertain the rate at which the trophic disturbances pass over the lateral-line nerve of the catfish, we cut this nerve on one side of the fish and then at a later period on the other side. After some twelve days when extensive degeneration of the medullary sheaths had set in, preparations of both nerves were made at the same time and a degree of degeneration shown by the anterior or proximal region of the second nerve to be cut was sought for on the first nerve. These regions of agreement would be expected of course to occupy different positions on the two nerves, posterior on the nerve first cut as compared with the second. The antero-posterior distance between the two regions of agreement must represent the length of nerve traversed by the trophic disturbance, as represented by degeneration, in the interval between the time of the first and of the second cutting. Rather to our surprise this distance proved to be very short, and it was only when the time interval was three or four days that satisfactory comparisons could be made. On the basis of a number of such tests it was ascertained that the rate at which the trophic influence passed over the lateral-line nerve was an extremely low one, approximately two centimeters per day, a rate which at once puts this type of transmission entirely out of the category of true nerve impulses and into a class by itself. That this rate is

probably very much more rapid in warm-blooded vertebrates than in cold-blooded ones is seen from the fact that complete nerve degeneration occurs in mammals in three to four days whereas it requires five to six times that length of time in cold-blooded forms. Nevertheless, even in instances where this process appears to be most rapid it falls far behind the rate of ordinary nerve transmission. Thus the lowest rate for such transmission, and this is in the nerve-nets of animals like the sea-pansy, is only seventy to eighty millimeters per second. Such a rate, however, is approximately 320,000 times that of the trophic influence. Hence there is good reason to believe that nerve impulses and trophic transmissions are fundamentally distinct.

The nature of the trophic influence that passes thus slowly over the lateral-line nerve of the catfish can be stated only hypothetically. Its great slowness indicates that it is not to be compared with the nerve impulse proper. Interference with it, as already pointed out, may result in the degeneration on the one hand of the nerve itself and on the other of its appended sense-buds. Of the five explanatory hypotheses that have been offered at various times for such conditions in general that which seeks to explain them as due to the inactivity of the parts concerned can have no application to the present instance. For when the lateral-line nerve is cut, the sense-buds and the distal portion of the nerve itself are left undisturbed and nerve impulses must continue to originate in the buds and pass over the attached portion of the nerve as usual. Hence if normal activity were all that was necessary to keep the peripheral portion of this mechanism intact, it should, under such conditions, remain so. But as a matter of fact under these conditions, as has been clearly shown, it degenerates completely. Hence normal activity of itself is insufficient to maintain the integrity of the nerve fibers and appended sense-buds of the lateral-line system.

Nor is there any evidence that after cutting the lateral-line nerve disturbances in the reflex protective mechanism or in the vasomotor system ever set in, as is so abundantly seen when the trigeminal nerve to the vertebrate eye is severed. Such disturbances, moreover, would scarcely be expected to occur in the lateral-line system, for the ordinary sensory nerve fibers to this system and to the adjacent skin as well as its vasomotor fibers do not find their way hither over the lateral-line nerve but over the numerous spinal nerves on the flanks of the fish. Hence there is no reason to suppose from the anatomical disposition of the parts that cutting the lateral-line nerve would cause in any way disturbances in the protective or vasomotor mechanism, and in fact none is seen. We, therefore, conclude that the degeneration of the lateral-line sense-buds in consequence of cutting their nerve is in no way due to interference with protective or with vasomotor factors.

In all our work we have seen no reason to assume specific trophic fibers. The lateral-line nerve is composed of a remarkably homogeneous group of elements. They give no evidence whatever of falling into histological subgroups, and physiologically the nerve has never been shown to be anything other than a purely sensory system. It is our opinion that all its nerve fibers are strictly sensory. In respect to specific trophic fibers we agree with Verworn ('99) and with Bayliss ('24), both of whom after a general survey of the subject declared against them. All nerve-fibers are trophic and transmit trophic influences either in the same direction as they do nerve impulses or in the reverse. In this respect double conduction must be a usual and normal phenomenon. How this can be accomplished has already been suggested (Parker, '29a, '29b).

What the nature of the trophic influence sent over nerve-fibers is can not be stated definitely. That it is something that passes over the fibers with extreme slowness seems probable from the results of our measure-

ments. Such a rate suggests the percolation of material through nerve-fibers, small as their cross-sections may be, rather than the spread of ionic readjustments such as represent the true nerve impulse. The physical difficulties concerning such a percolation has already been pointed out by Hardy ('27), but as every histologist who has worked with fresh nerves and stains knows, solutions of such materials do make their way into the axis-cylinders of fibers at the nodes of Ranvier and percolate slowly along the core. We, therefore, are of opinion that the so-called trophic influence in nerves is due to a substance or substances that emanate probably from the nucleated portion of the neurone and make their way over the whole length of the nerve-fiber, at the slow rate which we have determined roughly for the lateral line of the catfish. These substances we believe to be essential to the normal metabolism of the nerve-fiber itself and directly or indirectly to that of such end-organs as the lateral-line buds. A similar opinion, at least so far as the last step of this process is concerned, has already been expressed in the case of the taste-buds and their nerves by Olmsted ('20) and by May ('25).

Our conclusion, based on what we have seen in the relation of the lateral-line nerve to its sense-buds, is that *all* nerves are *trophic nerves* and that their trophic influences are not limited to one neurone but are the means of binding nerve-elements together. In this way the receptor cells in sense-buds depend upon their associated nerve-fibers. The control emanates from the nucleated part of the neurone and spreads probably in the form of one or more hormone-like substances throughout the nervous unit in which it originated, binding the part of this unit together and bringing it into close organic relations with others. In this last respect we touch upon the humoral agencies in nervous action, nervous secretions and the like (Parker, '31), a general field long ago cultivated by Ramón-y-Cajal in his hypothesis of the chemical interaction of neurones.

LITERATURE CITED

Bayliss, W. M.
1924. "Principles of General Physiology." London, 882 pp.

Bethe, A.
1903. "Allgemeine Anatomie und Physiologie des Nervensystems." Leipzig, 487 pp.

Brockelbank, M. C.
1925. "Degeneration and Regeneration of the Lateral-line Organs in *Ameiurus nebulosus* (Les.)," *Jour. Exp. Zool.*, 42, 293–305.

Fleischhacker, H.
1927. "Die tropischen Einflüsse des Nervensystems," Bethe, A., G. v. Bergmann, G. Embden, and A. Ellinger, Handb. norm. pathol. Physiologie, 10, 1149–1178.

Hardy, W. B.
1927. "Molecular Orientation in Living Matter," *Jour. Gen. Physiol.*, 8, 641–643.

May, R. M.
1925. "The Relation of Nerves to Degenerating and Regenerating Taste-buds," *Jour. Exp. Zool.*, 42, 371–410.

Olmsted, J. M. D.
1920. "The Results of Cutting the Seventh Cranial Nerve in *Ameiurus nebulosus* (Les.)," *Jour. Exp. Zool.*, 31, 369–401.

Parker, G. H.
1929a. "What are Neurofibrils?" AMER. NAT., 43, 97–117.
1929b. "The Neurofibril Hypothesis," *Quart. Rev. Biol.*, 4, 155–178.
1931. "Humoral Agents in Nervous Activity with Special Reference to Chromatophores." Cambridge University Press. (In press).

Verworn, M.
1899. "General Physiology." Trans. by F. S. Lee. London, 615 pp.

SPONGES AND BIOLOGY[1]

PROFESSOR H. V. WILSON
UNIVERSITY OF NORTH CAROLINA

IF the sponges could express themselves, as is the desire of so many of us to-day, I think a well-speaking sponge might address biologists somewhat in the following fashion: "I realize that we are not as widely known as some others and yet I feel that our family memoirs show we are not an uninteresting race. Dujardin, as early as 1838, when the cell generalization was in the making, wrote of our active amoeboid elements. Lieberkühn, in 1856, told the world that we had eggs, much like those of dog or man. At an appropriate time, when the evolution idea was acting like a fresh ferment, Haeckel, in 1872, described the beautiful series of forms grading up from simplicity to complexity, which the genius of the sponge race had realized in one of our tribes, the Calcarea. Oscar Schmidt had already begun ('62) his series of memoirs telling of our efforts, especially in the manufacturing of spicules, to present clearly this idea of evolution to the minds of men. These earlier recorders of our ways and our achievements have been followed by a line of modern biographers, led by F. E. Schulze and W. J. Sollas, all of whom testify to our interesting features. We are then not an unknown people.

"In writing of us, some biographers have been drawn by temperament or by their conceptions of what is most important to dwell especially on our cellular behavior. In this way they have made us admired, although perhaps at too great a distance, by the physiologists. Others, and the majority, like the great writers just mentioned, have been led to describe the *results* of our activities, the builded and finished structures, and in dealing

[1] Paper read by invitation before the New Orleans meeting of the American Society of Zoologists, December, 1931.

with these wonders of morphology many have shown an elegant technique that is well worthy of us. It will be admitted that the human study of classification, in its search for evolutionary affinities, owes us much."

Thus the sponges! Let us see what they really can do in the way of some cellular activities. And first, in the elaboration of reproductive bodies.

The observable facts lead us all to conclude that sponges may develop from eggs. For in the body of the parent we find a complete series of stages from egg to ciliated larva, and no indication that the larva is formed from any other body. Similarly, in certain monaxonid and hexactinellid sponges the observable facts, if any conclusion at all is to be drawn from them, lead with equal strength to the conclusion that a ciliated larva quite like that developed from an egg may sometimes be developed from a mass of undifferentiated mesenchyme cells, the so-called archaeocytes. This conclusion seems well established, since the facts are abundant, may easily be observed with precision and have been recorded in full by two observers for two groups (H. V. Wilson, '91, '94; I. Ijima, '01, '03), and in part by F. E. Schulze, unsurpassed in his knowledge of sponges ('04).

If we reason from these and other facts we must conclude that in the sponge cells, commonly now known as archaeocytes, we have elements in which there lie potentially all the characters of the species. If a cell of this kind is properly fed by follicular slave cells, it shows that it has the power to become an egg, which in due course becomes a larva and then a sponge. When, on the other hand, it combines, as a mere cell, with its fellows, the aggregation shows that it can do all, including the making of a larva, that the egg can. In some forms, the spongillids, the larva-forming power of the archaeocytes is exhibited only when they develop into eggs. For when they aggregate forming multicellular masses or gemmules, these bodies develop directly into sponges. The larva-forming power is here inhibited.

With increase of knowledge and improved technique we may hope some day to be able so to activate these cells as to turn them at will into eggs, sperm, larva-forming gemmules, gemmules with direct development or specialized tissue cells, into which, I may remark, they have also the power of transforming. No one as yet has been able to do this. Nevertheless, some progress, if not much, has, I think, been made toward this goal.

We have learned that if silicious sponges are kept in confinement they at once begin to adapt themselves to the unusual and unfavorable environment. The oscula and pores gradually close and the current of water passing through the body is reduced and finally stopped. All ordinary feeding and aeration of the interior is now at an end. In this state of danger what we would think of as a "*sauve qui peut*" policy is adopted. Organization is abandoned. Canals close and cells give up their specialized callings, as Metschnikoff first showed in 1879. A movement begins toward points that are presumably advantageous. Here cells accumulate and form solid masses of a relatively simple structure. Elsewhere death strikes the struggling mass of elements, the actions of which are physiologically reducible, it may be, to tropisms. We thus have left, in the best cases, a coherent body of skeleton and débris, lodged on and through which are many brightly colored compact little masses, often more or less spheroidal and in the neighborhood of a millimeter in diameter. They suggest at once the gemmules found in many localities at the end of summer scattered through the skeletal remnants of dead or dying spongillids. Like them, they are resistant, and when transferred to their natural environment they again, like gemmules, quickly transform into functional sponges.

What has been the actual cellular behavior that has led to the production of these regenerative masses? The process, in the gross one of simplification, has been studied by several (Maas, Müller, H. V. Wilson), but it still remains a promising field of research. The archaeo-

cytes are certainly leaders in what goes on. Since the process is linked in many ways to what happens when sponge cells are forcibly and suddenly separated and then allowed to reunite, I pass over it here. I would say, however, that what we already know shows that the simplification of the sponge may stop at more than one point before it has gone as far as it can in its downward course and be succeeded by an upward process of organization which leads again to the typical sponge structure (Wilson, '07a). It should be mentioned that the gross aspects of this process were described long ago in France by Laurent ('44), and in Germany by Lieberkühn ('57), both of whom observed that when sponges were kept in confinement they degenerated, leaving masses of tissue that had regenerative power. Their brief accounts (Müller, '11b), interesting as they were physiologically, had been lost sight of by 1907, when the phenomenon was redescribed as a new method by which sponges might be grown (H. V. Wilson, '07a). Otto Maas at about the same time ('06) had produced similar bodies in calcareous sponges and thought that they might be regenerative, as indeed he later found they were ('07).

The study of cell behavior in reducing sponges, leading to the formation of regenerative masses, very naturally led to more direct experimentation on the cells. And so I was led to find out in the summer of 1907, at the Beaufort Laboratory of the Bureau of Fisheries, that silicious sponges in the normal state can easily be broken up into their constituent cells which stream out into the water like clouds of echinoderm eggs, and, if mechanically aggregated and sown with a pipette on glass or a mollusk shell, will transform into functional sponges (Wilson, '07b, '11a). Some few years later I had the pleasure of learning that the same method is applicable to hydroids (Wilson, '11b). Many now in the past 20 years have used and amplified this method of growing sponges and hydroids. The experiment is easy and the result always rather pleasantly surprising.

But when the dissociated sponge cells assemble and form the regenerative mass, usually now designated as the reunition mass, what share, if any, does each kind of cell take in building the new sponge? This has proved to be a question not so easy to answer and the conclusions reached by the investigators have differed in some important details. There are indeed several conceivable answers to our question. Let us formulate these conceivable answers themselves as questions.

We may then ask: (1) Is there a permanent specification of cells in a sponge, possibly into epidermal cells, epithelial cells lining the canals, collar cells, cells that secrete spicules, cells that secrete the horny matter of the skeletal fibers, and into the other distinguishable kinds of cells in the sponge mesenchyme? And do these several kinds of cells, after dissociation, crawl back into their proper places in the reunition mass? Or (2) does a wide-spread, perhaps universal, process of dedifferentiation occur, the various kinds of cells resorbing their special differential cytoplasmic features and passing into a generalized embryonic state from which differentiation again starts out as from a group of blastomeres or a gemmule? Or (3) does rejuvenescence of another kind take place, the elementary, undifferentiated, proletarian cells, the so-called archaeocytes, engulfing and absorbing the more specialized elements, this process of phagocytosis gradually leading to the formation of a quasi-embryonic mass like a gemmule? Or (4) do the archaeocytes, without exercising the open brutality of phagocytosis, remain as the only persistent elements simply by inducing a condition, or perhaps merely assisting in the French sense at the oncome of a condition, in which other cells die and disappear quietly by cytolysis? Or (5) finally, is the whole phenomenon a composite of such processes?

I have recently ('30) gone over this matter with Dr. J. T. Penney. We find (1) that my original surmise, *viz.*, that a permanent and general cellular dedifferentiation

takes place, is not supported by the observable facts. Nor (2) is there any extensive phagocytosis. Nor (3) can it be said that the differentiated cells of the sponge return to their proper places resuming their former characters. It is true, we find, that the collar cells do this, a fact that has been affirmed (Huxley, '11) by some and denied by others (Müller, '11a; Galtsoff, '25), but they seem to be the only cells behaving in this way. The nucleolate mesenchyme cells, the so-called archaeocytes, evidently give rise anew, as in a gemmule, to many of the specialized cell groups. A hitherto overlooked, or misconceived, type of mesenchyme cell, one of much interest in considering regenerative phenomena, was discovered. Like the archaeocytes it is amoeboid but much more actively so. Unlike them it has, in the form (Microciona) studied, distinctive cytoplasmic granules and a characteristic, non-nucleolate nucleus. It can then be distinguished with certainty. These cells are abundant and very active. They quickly crowd to the surface of the reunition masses and there unite to form a protective covering, the permanent epidermis. The indications are that cells of the same kind form the lining epithelium of the canal system, a layer essentially like the epidermis. It seems very probable that such cells in the normal sponge body perform the function of quickly healing over a wound. Wounds are certainly very quickly covered over with new epidermis, and this we know is formed by amoeboid mesenchyme cells which come to the cut surface and there unite (H. V. Wilson, '10). These non-nucleolate mesenchyme cells are not totipotent like the archaeocytes. Nevertheless, they are regenerative but their regenerative power is limited. They can not do all, like the slower archaeocytes, but what they can do, they do very quickly. They suggest that there is more functional organization in the cell-community which we call a sponge than one would anticipate.

We may say, then, that in the formation of a new sponge from dissociated cells, unspecialized totipotent

elements, together with regenerative elements of specialized power, reestablish, as a new formation, the bulk of the sponge. Nevertheless, the collar cells, carried over from what we may call the parent, being on hand, are made use of as such. The method of restoration employed is a combination method, one well adapted to reestablish the sponge structure quickly.

The recorded data indicate that the same method is probably sometimes, *i.e.,* at a certain level, employed in the case of the reduction masses already mentioned, but that in these the process of reduction may go farther in the direction of establishing a homogeneous, "harmonious-equipotential" tissue. I thought at one time this was probably accomplished by fundamental cellular dedifferentiation, all the cells passing back into a simplified embryonic state and so surviving, and I still think this idea should be kept in mind in renewed research in this field. Others (Maas, '10; Müller, '11b) have, however, reached the conclusion that the homogeneous state is arrived at by phagocytosis, the persisting elements being certain amoeboid cells which devour the others. One would think that, if this is so, the eventually persisting cells would be the totipotent archaeocytes and Müller concluded that perhaps this was so in the case of Spongilla. If, however, Maas' tentative conclusion for the Calcarea is right, these cells are for the most part not archaeocytes but specialized tissue cells, *viz.,* pore cells, that have dedifferentiated into a morphological and physiological state like that of archaeocytes. This, in its emphasis on pore cells, which in Maas' forms, the Sycons, have not been found by every one, is of course surprising, and Maas admits the possibility that in the end the dedifferentiated pore cells may be devoured by the real primitives, the archaeocytes. The field, as can easily be imagined, bristles with technical difficulties as do related ones, where also the need is to follow the later history of particular kinds of cells which become dedifferenti-

ated, so far as to lose their distinctive morphological characters, and which unite syncytially.

In this sketch of methods of propagation in sponges we may note the following outstanding facts. (1) The cytoplasmic body of a sponge cell can not be looked on as a persistent unity, *i.e.*, a permanently individualized mass. Fusion with other cells is too frequent and too complete to allow of such a view. (2) Morphological dedifferentiation of cells occurs, but at any rate in some cases, that of the collar cells, for instance, it is not final but temporary, the distinctive features being regained. (3) It has yet to be proven that sponge cells ever dedifferentiate into a regenerative, embryonic condition, although the dissociation phenomena in hydroids lends some support to the view that this is possible. (4) Adaptive types of cellular behavior, which tend to insure survival, are called out under adverse conditions, as in the reduction and dissociation phenomena. (5) Certain cells are totipotent, egg-like as it were, and may hence develop in any of several directions, as in the case of archaeocytes, while other cells may have only a special though very quickly exercised regenerative power, as in the case of the non-nucleolate mesenchyme cells.

The retention by cells of their essential nature, even after complete morphological dedifferentiation, is illustrated, as said, by the dissociated collar cells. A parallel, not complete by any means, is found in the metamorphosis of the solid ciliated larva of our common silicious sponges. I have recently studied anew at the Naples Station this corner of comparative embryology, that has not been touched in a descriptive way for many years, and I find that the ciliated cells covering the surface of the larva are in some way drawn into the interior, losing their shape, distinctive granules and cilia and becoming syncytially interconnected with other cells. Out of this syncytium reemerge well-defined blocks of cytoplasm surrounding the original epithelial nuclei, and these become the collar cells. Yves Delage's discovery ('92) was,

then, in the main a real one, although there seems to be no reason for believing that the cytoplasm of the collar cell is the same mass that surrounded the nucleus of the epithelial cell. Delage, to be sure, described a process of temporary phagocytosis whereby this end was achieved. In this process larval epithelial cells were engulfed by amoeboids, in which they remained for a time in a symbiotic state but from which they were eventually freed to become the collar cells. I believe with Maas ('93) and Nöldeke ('94) that Delage was here in error and that what he saw was not symbiosis but serious internecine conflict, that is, the incorporation by amoebocytes of some of the epithelial cells, perhaps those that could not, for one reason or another, be used in morphogenesis, and the actual digestion of the nuclei, at any rate, of the ingested cells.

As to the main point, however, Delage was right and passing over for the present the question, how much of the cytoplasm of the epithelial cell goes into the collar cell, we may say that in a general way larval epithelium cells do become collar cells. A parallel is thus offered, although, as I have said, an incomplete one, to the behavior of the dissociated cells. For the larval cell after its period of submergence regains in some degree its original nature, coming out with an independent and somewhat elongated shape and a cilium. On the other hand, it gets what it did not have before, a collar, and there are in the cases I have studied (Mycale, syn. Esperella, Esperia) certain distinctive cytoplasmic characters which it does not recover. It reemerges then into active individual life like, but yet unlike, its old self.

I may incidentally add that I find that Goette was correct in concluding ('86) that the definitive epidermis of the sponge is formed from peripheral cells of the inner larval mass. Delage and Maas added to this knowledge, but in some way the original discovery by Goette has been nearly lost sight of (Nöldeke, '94).

I may close this sketch of events in the cellular life of sponges by considering for a moment the subject of embryogenic variation. It is now clear, and I seem to have been the first to point it out ('91, '94), that in the metamorphosis of the monaxonid sponge larva the flagellated chambers may be formed in two ways in the same species, indeed in the same local race, and perhaps even in the same individual. In the first place they are formed, as I have just said, from the larval epithelium cells that are drawn into the interior, strictly speaking, and not going beyond the known facts, from the nuclei of these cells and the cytoplasm which surrounds them after they have entered into the general syncytium. In my earlier work I observed this method but failed to discover the true origin of the little cells that group themselves around an intercellular space and transform into collar cells. Nevertheless, I recognized and recorded it as a method of chamber-formation distinct from the one I now turn to.

In this, the second, method the collar cells are formed from comparatively large mesenchyme elements, formative cells or archaeocytes, which divide. The two methods are sharply separated, far more so than I imagined when I first recorded them. For one is not derived from the other, as I had thought. Delage's discovery of the true nature of the first method makes this plain, and this method is clearly the basic ontogenetic one.

The two methods are practiced in the metamorphosis of other sponges than those for which I recorded them in 1891. Evans ('99), for instance, eight years later found both to occur in Spongilla. With this diversity of morphogenetic method established, a field perhaps is opened to experimental analysis.

The method of forming flagellated chambers from archaeocytes, while a secondary one in the metamorphosis, is used in a good many larvae. This is probably (Weltner, '07: 277) also the method of forming new chambers during ordinary post-larval growth, and is the method used in the development of a spongillid gemmule

and in some cases, at any rate, in regeneration from a reduction body (Maas, Müller). It is then the common method of later life, and its presence in the metamorphosing larva may be looked on as a precocious appearance. Why should it appear at this time? One can only speculate. It is clear that the larval epithelium cells have a hard time during metamorphosis. Some are lost at the surface, as I find occurs even in healthy larvae. Others are phagocytosed by amoeboids. The survivors form the chambers. Why such slaughter? Is it just that there are many more epithelial cells in the larva than are needed for the formation of chambers? However this may be, given the slaughter, it may well happen that sometimes it goes too far, and not enough epithelium cells (nuclei) are left for an adequate equipment of chambers. In such an emergency the archaeocytes are called on ahead of their time and they save the day. Their precocious coming into action may then provisionally be looked on as an adaptive measure carried out by the larva, an instance of that regulation of embryonic processes which insures that an attempt, at any rate, shall be made to form an embryo, if not in one way then in another. Cases of this regulative action more familiar to many are afforded by the spina bifida embryos of fish and amphibia.

This power of self-adaptation (self-regulation of W. Roux, '14) in an embryo is certainly one of the remarkable phenomena in nature. One can not but ask, although one can not answer the question, does it ever lead to hereditary change in embryogenic processes, to the development of a new hereditary way of modeling out an embryo from egg or mass of cells? To be sure, there are those who would say the power of self-adaptation is only an illusion, that in a case of this kind we have nothing more than a sequence of simple physico-chemical events, each mechanically necessitating that which follows, as when fallen leaves whirl about and collect in certain configurations. Which causes one to reflect that after all the

out-and-out mechanist is less realistic than he might be, for it really seems necessary at the present time to recognize self-adaptation as an actuality and advisable to learn both more cases and more about them.

LITERATURE CITED

Delage, Yves
1892. "Embryogénie des Éponges," *Arch. Zool. exp. et gén.*, sér. 2, 10.

Evans, R.
1899. "The Structure and Metamorphosis of the Larva of *Spongilla lacustris*," *Quart. Jour. Micr. Sci.*, 42, n. s.

Galtsoff, P.
1925. "Regeneration after Dissociation (an Experimental study on Sponges)," I and II, *Jour. Exper. Zool.*, 42.

Goette, A.
1886. Abh. z. Entw.-gesch. d. Tiere III. "Unters. z. Entw.-gesch. von *Spongilla fluviatilis*."

Huxley, J. S.
1911. "Some Phenomena of Regeneration in Sycon," *Phil. Trans. Roy. Soc. London*, B, 202.

Ijima, I.
1903. "Stud. on the Hexactinellida," III, *Jour. Coll. Sci., Imp. Univ. Tokyo*, XVIII.

Maas, O.
1893. "Die Embryonal-Entwicklung u. Metamorphose d. Cornacuspongien," *Zool. Jahrb. Abt. Anat. u. Ontog.* VII.

Müller, K.
1911a. "Das Regenerationsvermögen d. Süsswasserschwämme," u. s. w., *Archiv. Entw. mech.*, 32.
1911b. "Reductionserscheinungen bei Süsswasserschwämmen," *ibid.*, 32.

Nöldeke, B.
1894. "Die Metamorphose d. Süsswisserschwämmes," *Zool. Jahrb. Abt. Anat. u. Ontog.* 8.

Roux, W.
1914. "Die Selbstregulation," *Nova Acta. Abh. kaiserl. Leop.-Carol. deutsch. Akad. Naturf.* C, Nr. 2.

Wilson, H. V.
1891. "Notes on the Development of Some Sponges," *Jour. Morph.*, 5.

Wilson, H. V. and J. T. Penney
1930. "The Regeneration of Sponges (Microciona) from Dissociated Cells," *Jour. Exp. Zool.*, 56.

(Other references made in the body of the paper are included in the literature list given in Wilson and Penney, 1930).

GROWTH RATE AND HORMONE THRESHOLD WITH REFERENCE TO PHYSIOLOGY OF DEVELOPMENT[1]

PROFESSOR FRANK R. LILLIE
UNIVERSITY OF CHICAGO

WILHELM ROUX ('95) defined the program of *Entwicklungsmechanik* ("physiology of development," as we should name it) as the resolution of developmental processes into simpler, but still complex, physiological components, and the analysis of the physiological components into really simple ones, "which may be identical with those which underlie inorganic or physico-chemical processes." This definition implies that nothing is left behind in the process of analysis, and that by summation or synthesis we could again reconstruct the whole from the parts. The implications of such a philosophy of biology are so profound that I have never felt satisfied to accept Roux's definition of the program; I prefer instead the definition that the aim of "physiology of development" is to discover the mechanisms of control of developmental processes. This sets us on the way to the discovery of mechanisms in development, but it does not imply a synthesis of development from the mechanisms; on the contrary, it definitely negatives such an attempt; and it reduces the status of physiology of development from "the most difficult problem which the human intellect has attempted to solve," as Roux characterized it, to a reasonable undertaking of a physiological order.

I shall present to you certain considerations involving hormone action in relation to growth rate as one of these mechanisms of control. Hormone action has been correctly conceived as organismal in the sense that all grow-

[1] Paper read by invitation before the New Orleans meeting of the American Society of Zoologists, December, 1931.

ing parts of the organism must be equally exposed to its influence. It has, hence, been difficult to conceive how in development it could exert localized, mosaic-like effects; and yet this is a rather conspicuous feature—witness, for instance, the near-specific effect of the sex hormones! Although this had to be granted, yet when it came to the interpretation of the existence side by side of male and female characters in the same individual, as in gynandromorphs of birds, it was felt that an explanation on the basis of the action of sex hormones was almost necessarily excluded, and that some dislocation of genes in accordance with known mechanisms of gene action must be invoked.

It is readily shown that *intermittent* hormone action may produce very precisely localized mosaics of male and female patterns in growing feathers (Domm, '27; Juhn and Gustavson, '30). The purpose of the present paper is, however, to show that, in addition, even with constant action, a variety of similar mosaic effects can be produced, which are dependent on the principle of thresholds of reaction varying from part to part.

The French investigator Pézard ('22) was the first to lay emphasis on the principle of thresholds in hormone action, and he even used it to explain bilateral gynandromorphism in the plumage of birds, by the assumption that the right and left sides of the body in such cases possess different thresholds for the female hormone in the growing plumage. I regret that I overlooked this interesting theory of Pézard's in my recent paper on "Bilateral Gynandromorphism and Lateral Hemihypertrophy in Birds" ('31). Pézard, however, did not demonstrate that unequal thresholds existed in the plumage, nor did he attempt an analysis of the theory.

I. The first evidence that varying thresholds exist in the plumage of fowls and that the different thresholds possess a simple physiological basis came as a result of the work of Juhn and Gustavson in my laboratory

in 1930, and of their collaboration with Dr. Gwendolen Faulkner in 1931.

They showed that different feather tracts of the brown Leghorn fowl have inherently different growth rates, and exhibit correspondingly different thresholds of reaction to the female hormone. It was thus possible to produce birds with female feathers growing in the saddle region and male feathers in the breast region by keeping the amount of female hormone injected above the threshold of reaction of the saddle feathers, which have a lower rate of growth, and below the threshold of reaction of the breast feathers, which have a more rapid rate of growth. In the case of concentration above the thresholds of all growing feathers, an interruption of injections would record the reversion to the male type earlier in the breast than in the saddle, owing to reduction of concentration of the hormone in the blood first passing the threshold for the breast. If injections were renewed soon enough, the breast feathers became gynandromorph, while the saddle feathers remained pure female, owing to the fact that excretion had not reached the lower threshold level of the saddle before the new supply of hormone became effective. In all cases the threshold of reaction exhibited an increase in correspondence with increase of rates of growth.

II. In the individual feather the same principle holds (Lillie and Juhn, '32), and it can be applied to the explanation of some important principles of feather pattern. One of these principles comes out very prettily in the patterns that it is possible to produce in saddle and hackle feathers by injections of thyroxin. The saddle and neck hackle feathers of the brown Leghorn react to injection of c.p. thyroxin by deposition of black pigment, and by extension of barbule formation, which is normally confined to the bases of the barbs of these feathers, towards the apices of the barbs, and even to their extreme tips. In the case of the saddle feather, with a single injection of 0.5 mg of thyroxin into a capon weighing

1,800 grs, the pattern produced is a narrow black spindle centering on the rhachis of the feather. The spindle records at its apical end the first effective concentration of excess thyroxin in the blood; its widening records rise of concentration with absorption, and its taper to the basal end records excretion to normal. The threshold of reaction thus rises from the base of the barbs towards the apex. With a single injection of 1 mg the spindle is wider, inasmuch as successive apical thresholds are passed; the widest part of the spindle in this case is above its center, recording a more rapid process of absorption than of excretion. Single injections of 1.5, 5 and 10 mgs of thyroxin produce patterns that finally reach the margin of the feather, all threshold levels being exceeded in the last case. The pattern in the successive cases approaches and finally reaches the form of an inverted equilateral triangle, the process of absorption being recorded only by a slight anterior projection from the center of the base.

Repeated symmetrical patterns can be recorded on either saddle or neck hackle feather, by interrupted injections properly spaced. In the production of these patterns the invariable principle is that threshold of reaction rises from base to apex of the barbs.

Lillie and Juhn ('32) have shown in a very detailed way that this gradient of thresholds corresponds to a gradient of growth rates of the successive levels of the individual barbs. The apical portion of the barb, which is first formed in development, has the most rapid growth rate and this diminishes constantly towards the base.[2]

Patterns of similar form, though different in detail, may be produced by injections of female hormone. Thus two substances demonstrate equally well that the principle of a direct relation between rate of growth and

[2] This can not be adequately described without the figures which were used in the address. Those interested in this fundamental point in the physiology of development of the feather are referred to the paper by Lillie and Juhn ('32) published since this address.

threshold of reaction holds as well for parts of a single feather as it does for feathers of different growth rate.

We have spoken of feather patterns of axial origin dependent for their form on the principle of the relation between threshold of reaction and rate of growth. Another principle is, however, involved in the determination of the patterns, *viz.*, the principle of *rate of reaction.* The amount of effect produced in a given time is naturally greatest in regions of most rapid growth rate. Not only is this so, but the latent period before reaction begins, which is very considerable, ranging from 24 to 48 hours or more, is much shorter in regions of greatest growth rate. It may therefore happen in a case where the determining agent reaches a maximum concentration in a very brief space of time that its effect is confined to the margin of the feather. It is possible to produce such marginal marks experimentally. Indeed, any pattern produced by a determining agent operating at a concentration above all the thresholds must begin at the margin in point of time. The pattern produced in any case depends on the form of the curve of concentration with reference to the two principles of threshold and rate of action. Patterns may thus be of marginal as well as of axial origin.

III. Asymmetrical patterns also may be determined by differences in rates of growth on the two sides of the plane of symmetry. In the case of feathers of the wing-coverts we have been able to secure reaction to the female hormone confined to one side of the vane. It is interesting to note that on opposite sides of the body such asymmetries are mirror images of one another. The same principle appears to apply to bilateral gynandromorphism of the plumage of the entire bird; the writer (Lillie, '31) has shown that this condition is associated, frequently at least, with hemilateral hypertrophy, and that the male plumage is confined to the hypertrophied side. The assumption is that the hypertrophied side has a more rapid rate of growth with a correspondingly

higher threshold of reaction to the female hormone, so that, with the defective ovarian conditions obtaining in such cases, only the lower threshold of the more slowly growing side is attained by the hormone; the plumage of the other side would thus remain asexual, *i.e.,* male in type.

IV. Returning to our more general considerations, we can see how a simple physiological mechanism of control (such as a hormone circulating in the blood), operating on a developing system, the feather germ, which possesses gradients in growth rates, can produce astonishing variety of results in structure and pattern; and we can thus begin to understand one of the ways in which the almost infinite varieties of natural feather patterns may have been guided in their evolution. Other mechanisms of control of the feather type also exist in the genetic composition of feathers and in special innate properties of the different feather tracts, which constitute a mosaic of largely self-determined entities in other important respects. The physiological controls of which we have been speaking operate within limits set by these innate controls.

Two classes of phenomena are involved, *viz., threshold* and *reaction.* "Threshold" may be defined as quantity of condition necessary for occurrence of reaction. "Reaction" is the specific performance of a living system. These are related to rate of growth as follows:

(1) Increase of threshold is directly proportional to increase of rate.

(2) The latent period is inversely proportional, and the amount of reaction is directly proportional, to increase of rate; in other words, the time necessary for onset of reaction, *i.e.,* the latent period, is diminished, and the amount of reaction is increased with increase of rate.

Rate of growth is thus the determining factor (1) with reference to threshold, (2) with reference to duration of

the latent period, and (3) also with reference to the amount of reaction within a given period of time.

On the basis of ordinary physiological, or indeed chemical, principles it is easy to understand how the latent period should be cut down, and the amount of reaction within a given period of time be increased, with increase of growth rate. But it is not so easy to understand why a more slowly growing part of the system should react to a lower concentration of the hormone than more rapidly growing parts; in other words, have a lower threshold. This determination does not, however, stand alone.

In Child's experiments (*cf.* Child, '28) dealing with differential susceptibility along the axial gradient, disintegration in lethal solutions begins at the high point of the gradient and progresses towards the low point; in such an experiment the concentration is above the lethal point for all levels, and the result therefore measures merely the rate of reaction—in this case disintegration. This is in agreement with our results with supramaximal hormone concentrations. When, however, in Child's experiments lower concentrations of the lethal agent are employed, the phenomenon that he calls differential acclimation appears; disintegration proceeds in the opposite direction; and the region of highest metabolic rate survives and may subsequently regenerate the missing parts.

This phenomenon is primarily differential *survival* rather than acclimation, and the result is that the threshold of survival is least at lowest rates and greatest at highest rates, thus in the same order as our threshold of reaction.

The field of study of differential susceptibility to various agents—chemical, pharmacological, physical agents, radiation—is a very large one. These have been studied mostly from the point of view of injury rather than stimulation. It is accordingly difficult to find investigations comparable to our own. There is, however, rather general agreement that there is some connection between

differential susceptibilities and rates of metabolism. Packard ('31), for instance, says with reference to the biological effects of short radiations, after considering apparent exceptions to such a relationship: "These instances, however, are not sufficient to outweigh the large amount of evidence which indicates that there is some connection between susceptibility and the metabolic rate, or some physiological condition closely bound up with it." Wertheimer ('30) believes that there is a direct relation between rate of growth and susceptibility to thyroxin, young rapidly growing individuals being much less susceptible than mature slowly growing individuals, a relationship which is in the same order as our observations on the feather germ.

Rather general application of the principles of threshold and rate of reactions with reference to growth rates would appear to be possible in physiology of development. The distribution of growth rates within the organism varies enormously with age, with reference to organs and regions, and with reference to symmetry, whether axial or bilateral. These subjects have been much studied of late. If the principles of relation between growth rate and threshold apply to all vital reactions, they must be factors in effects produced by all organismal mechanisms of control, such as hormones and genes. In growth rate, therefore, we may have one of the most important mosaic principles of development.

LITERATURE CITED

Child, C. M.
1928. "The Physiological Gradients," *Protoplasma*, 5, 447–476.

Domm, L. V.
1927. "New Experiments on Ovariotomy and the Problem of Sex Inversion in the Fowl," *Journ. Exp. Zool.*, 48, 31–174.

Juhn, Mary, and R. G. Gustavson
1930. "The Production of Female Genital Subsidiary Characters and Plumage Sex Characters by Injection of Human Placental Hormone in Fowls," *Journ. Exp. Zool.*, 56, 31–61.

Juhn, Mary, G. H. Faulkner and R. G. Gustavson
1931. "The Correlation of Rates of Growth and Hormone Threshold in the Feathers of Fowls," *Journ. Exp. Zool.*, 58, 69–111.

Lillie, Frank R.
1931. "Bilateral Gynandromorphism and Lateral Hemihypertrophy in Birds," *Science*, 74, 387–390.
Lillie, Frank R., and Mary Juhn
1932. "The Physiology of Development of Feathers. I. Growth Rate and Pattern in the Individual Feather," *Physiol. Zool.*, 5, 124–184.
Packard, Charles
1931. "The Biological Effects of Short Radiations," *Quart. Rev. of Biol.*, 6, 253–280.
Pézard, A.
1922. "La Loi du 'tout ou rien' et le gynandromorphisme chez les oiseaux," *Jour. de Physiol. et de Path. Gen.*, 20, 495–508.
Roux, Wilhelm
1895. "Aufgabe der Entwickelungsmechanik," *Arch. für Ent'mech.*, 1, 1–42.
Wertheimer, E.
1930. "Uber die Sonderstellung von Zellen mit Stärkster Wachstumstendenz im Organismus," *Pflüger's Arch.*, 225, 118–130.

SHORTER ARTICLES AND DISCUSSION

AN INTERNAL BUT NON-GENETIC CHARACTER AFFECTING WING PRODUCTION IN RESPONSE TO LIGHT IN AN APHID[1]

A STRAIN of the rose and potato aphid *Macrosiphum solanifolii* (=*gei*) collected near Woods Hole, Massachusetts, in July, 1928, proved to differ from any other strain of this species which I have reared in exhibiting a considerable range of color in the females. Some females were bright green, some distinctly yellowish, with various gradations between. This was not an ordinary case of fluctuating variation, with bright green and yellow the extremes and the mode somewhere in the middle of the range. The intermediate colors were far too few to warrant such an assumption. There was a mode in the yellow part and another in the green part of the range, with a trough between.

This color difference was not usually apparent in the nymphal stages, and was never marked in them. Differences were distinctly observable in young adults, and often became greater with age. That is, a young adult yellower than the average would often, perhaps usually, become still yellower during its adult life. However, a female that was yellower than the average as a young adult was never found to turn distinctly green with age. More than fifty yellowish adults were individually observed during their lifetimes with respect to this point, and most of them turned somewhat yellower, some of them became much yellower, but none became more green. The comparisons were made with Ridgway's "Color Standards and Nomenclature," as the objective standard, and all the changes observed in these fifty or more aphids were toward the greater wave-lengths.

Similar tests were made with young adults that were bright green. In them, the changes occurring with advancing age were much less marked than among the yellow females, and few of them showed any very appreciable change at all. What modifications did take place were, however, toward the green. Now and then a very old female, at a time when the body for some reason becomes somewhat shriveled, was distinctly blue-green. I

[1] Contribution from the Zoological Laboratory of the University of Michigan.

am not, however, inclined to regard this change toward the shorter wave-lengths as the same phenomenon as the small change occurring in a few of the plump females toward greener color, since such old blue-green individuals have been repeatedly observed in other strains.

The comparison just made could lead to the conclusion that bright green was the standard condition, from which a change toward yellow was rather readily made. A diminution of the yellow was, however, difficult and never very extensive.

The possibility that the color difference had a genetic foundation was tested by rearing more than twenty bright green females and an equal number of distinctly yellow ones, and recording the colors of their progenies. These were recorded by their Ridgway names at first, then converted into their numerical or other symbolic designations according to the Ridgway scheme. Only their numerical values need be used here, since these values (from 1 to 72) are the ones that relate to wave-length. Only healthy adult offspring were studied, the few that were rejected being rejected wholly on other grounds than color. All were among the first four days' output of their respective parents. All were taken at approximately the same age, which was about four days after becoming adult. Experience has taught that at that age any color differences that would arise were already marked, and using adults no older than about four days avoided confusion of their color peculiarities with color changes (like the acquisition of blue-green by old females mentioned above) due solely to old age.

The progeny of the twenty yellow females had a mean numerical color rating (relating to wave-length) of $26.93 \pm .065$, while the progeny of the twenty bright green parents had a mean color rating of $27.01 \pm .059$. The latter group was a little greener, but the difference was not quite equal to its probable error. There is probably no genetic difference, therefore, between the yellow and bright green aphids.

The response of these two types of aphids to light and darkness, with reference to wing production, was tested by rearing a group of each kind in continuous light, and an equal number in alternating light and darkness, after the manner of my earlier experiments (Shull, '28, '29). The length of the light period was 8 hours, the dark period 16 hours. Their progeny are represented in Table I.

TABLE I

Bright green parents				Yellowish parents			
Continuous light		Alternating light and darkness		Continuous light		Alternating light and darkness	
Wingless	Winged	Wingless	Winged	Wingless	Winged	Wingless	Winged
35	100	158	120	69	80	107	70
Per cent. winged	74.1		43.2		53.7		39.5

Owing to the very erratic occurrence of wings in aphids, it is not safe to draw conclusions from such small numbers as Table I includes. In obtaining larger numbers it was not possible, along with other experiments in progress that time, to continue all four of the lines represented in that table, so only those two which showed the least contrast in the first experiment were repeated. These were the groups reared in alternating light and darkness, and for them the experiment was repeated four times. The light and dark periods were this time necessarily slightly irregular, but were the same for both green and yellow females. The light period averaged about 7 hours, the dark period 17 hours. Table II shows the results of these four experiments.

TABLE II

Bright green parents			Yellowish parents		
Wingless	Winged	Per cent. winged	Wingless	Winged	Per cent. winged
184	66	26.4	283	89	23.9
72	111	60.7	88	112	56.0
163	129	44.2	115	69	37.5
141	139	49.6	163	80	32.9
560	**445**	**44.3**	**649**	**350**	**35.0**

While only one of these experiments shows a markedly different response in the offspring of green and yellow parents, the difference is in every case in the same direction. It appears safe to conclude that the green parents produced more winged offspring than did the yellow parents. Since the color was not of genetic origin, there must have been some physiological modification of a more or less permanent nature so far as the

individual was concerned. As pointed out above, it is somewhat more in keeping with the evidence to suppose that the yellow individuals were modified greens than that the greens were modified yellows. Yellowing, according to this supposition, was a condition which could easily increase in degree, but was not likely to disappear in a given individual once it got a start.

The nature of this modification is wholly unknown. It could be an infection, but there is no evidence of the presence of any other organism. The work of Ackerman (1926) on the physiology of wing development opens up many possibilities for speculation, but the present experiments offer no support for any proposed view. The fact that there was a modification seems, however, to be established.

A. Franklin Shull

LITERATURE CITED

Ackerman, L.
1926. "The Physiological Basis of Wing-Production in the Grain Aphid," *Jour. Exp. Zool.*, 44: 1–61.

Shull, A. F.
1928. "Duration of Light and the Wings of the Aphid *Macrosiphum solanifolii*," *Roux's Arch. f. Entwick. d. Org.*, 113: 210–239.
1929. "The Effect of Intensity and Duration of Light and of Duration of Darkness, Partly Modified by Temperature, upon Wing-Production in Aphids," *Roux's Arch. f. Entwick. d. Org.*, 115: 825–851.

AUTOPLASTIC TRANSPLANTATION OF GUINEA-PIG SKIN BETWEEN REGIONS WITH DIFFERENT CHARACTERS

At the suggestion of Professor S. Wright, experiments involving autoplastic transplantation of guinea-pig skin were undertaken to determine whether pigmentation, length and direction of growth of guinea-pig hair persist in a new environment or whether there is modification of these factors to conform to the surrounding pattern. Most of the animals used in the experiments showed spotting effects, and the technique consisted of an interchange of areas of skin of different color.

Although there are many records of skin transplantation, there are few cases in which it has been employed as a genetic method. Carnot and Deflandre ('96) and Leo Loeb ('97) found that black skin, transplanted in place of white on the ear, per-

sists, but that white, transplanted to a black area, became pigmented after a short period. This was due, according to Loeb, to migration of pigment granules from the surrounding black area into the graft skin. This was indicated by the fact that black skin transplanted to a white ear became paler for a time, while the surrounding area became pigmented. Their observations were restricted to changes in pigmentation of transplanted skin and were not concerned with the color of the hair.

Danforth and Foster ('29) used the method of skin transplantation in the fowl to determine the mode of action of genetic factors on the activity of the feather follicle. They found that color pattern of the feathers in the transplanted skin continues to be like that of the donor if the host is of the same sex. If of the opposite sex, the feathers became like those of individuals of the donor breed of the sex of the host. The hen feathering of male Sebrights, however, turned out to depend on the reaction of a local factor to the male hormone.

Their evidence also indicates that the difference in the rate of feathering in young Leghorns (rapid) and Rhode Island Reds (slow) is determined almost entirely by factors residing in the skin. Reciprocal grafts of the two breeds show that the time of the first production of contour feathers is constant for each breed and is not changed by transplantation. In the case of Plymouth Rocks, both local and correlative factors were indicated.

There are seven known series of allelomorphic factors acting on coat color in the guinea-pig (Wright '27). There was variation in five of the seven series of factors among the animals used in these experiments. Three of the series (S, s; A, a; E, e^p, e) effect color pattern, while the other series (C, c^k, c^d, c^r,

TABLE I

Pattern			Intensity		
	S	s		Black series (E)	Red series (e)
EA	Agouti	Agouti-white	CP	Intense black	Intense red
e^pA	Agouti-red	Agouti-red-white			
eA	Red	Red-white	Cp	Pale sepia	Intense red
			c^kP	Sepia	Yellow
Ea	Black	Black-white	c^kp	Very pale sepia	Yellow
e^pa	Black-red	Black-red-white			
ea	Red	Red-white			

c^a; P, p) modify the intensity of the color in the pattern. Table I shows the factors concerned with the regulation of the color pattern and intensity of the color of the hair of engrafted areas.

The spotting factor *s*, which acts to limit the colored areas to spots on a white background, was present in most of the animals used. A spotting pattern of black and yellow (within the colored areas) was often present, the intermediate allelomorph e^p of the series *E*, e^p, *e* being present in such cases. This series differentiates between black and yellow colored portions of the coat, some grade of yellow resulting if the animal carries *e* instead of *E*. The factor *A* is responsible for the agouti pattern in the dark areas of the coat, while if *a* is present instead, hairs in these regions do not show the agouti band.

The other two series of factors are concerned with modification of the intensity of the colors. The albino series (C, c^k, c^d, c^r, c^a) acts on both black and yellow, the allelomorphs of *C* reducing black to various grades of sepia and reducing red to yellow, cream, or white. The factor c^k was present in the sepia and pale sepia animals used, as well as in the yellow animals. The factor *p* also reduces black to sepia but has no effect on red.

Operations were carried out on young guinea-pigs, forty to fifty days old. The transplantations were all autoplastic and consisted of a transfer of rectangular areas of skin of approximately two square centimeters. The operation site was clipped, shaved and sterilized with 70 per cent. alcohol. The procedure in each case was that of an interchange of areas of skin of different color patterns. The grafts were held in place with narrow strips of adhesive tape, which was found to be as satisfactory as sewing the grafts in place. It was essential to hold the graft tightly in position, and this was accomplished by taping a small rubber sponge wrapped with sterile gauze in place over the graft. The graft was usually sufficiently incorporated within ten to fifteen days that the bandage could be removed.

It is seen from Table II that pigmentation of the hair was not modified in fifty-two successful grafts. The grafts were in every case observed for a long enough period as to leave little doubt that the color pattern is persistent. The animals were kept under observation for periods of between six and nine months, and there is very little possibility that observation for a longer period would have yielded different results.

TABLE II

		Type of transplant	Successful grafts	Color pattern unmodified
Color on white	Black series	Agouti on white	1	
		Black on white	4	
		Pale sepia on white	3	8
	Red series	Red on white	7	
		Yellow on white	1	8
White on color	Black series	White on agouti	2	
		White on black	4	
		White on pale sepia	2	
		White on sepia	2	10
	Red series	White on red	5	5
Dark color on red or yellow		Agouti on red	1	
		Sepia on red	1	
		Black on red	1	
		Sepia on yellow	1	4
Red on dark color		Red on black	1	1
Agouti		Back to belly	10	
		Belly to back	6	16

Four general types of transplants were made to test the persistence of color pattern. Transplantation of skin from a colored area (whether of the black or yellow series) to a white area showed that the hair follicles do not lose their pigment producing potentialities in an unpigmented area. Sixteen grafts of different color pattern, involving both red and black series, to a white area were all in agreement. L. Loeb reported that in cases in which black skin was transplanted to a white area there was a considerable increase in size of the black area due to migration of pigment into the surrounding white area. We did not observe any change in the area surrounding the grafts in any case.

The second type of transplant was that of white skin to a colored area. Even this type of graft, which was thought most likely to be subject to change, did not acquire a pigmentation after transplantation. Fifteen white grafts in a colored area indicated that white hair does not become pigmented. Accord-

ing to L. Loeb and earlier workers, white skin rapidly acquired a pigmentation when transplanted to a dark region, in some cases becoming indistinguishable from the surroundings. While some of our grafts of this type did become slightly darker along the border after four or six months, there were many cases in which such grafts remained unpigmented throughout the period of observation. In no case did the hair become pigmented.

Reciprocal transplants of areas of the red and black series of colors indicated that the original color is maintained in an area of different color.

The agouti guinea-pig shows a marked regional difference in color pattern parallel to a pattern in length of the hair found in guinea-pigs of all colors. Each hair of the back is black with an intense yellow, narrow, sub-terminal band, while each belly hair has a wide, terminal yellow band. There is a very distinct difference in length of the hair of the two regions, the hair of the back measuring 27–35 mm in length in comparison to that of the belly, which is 10–15 mm long. We interchanged pieces of skin of these two regions in order to determine whether these differences are persistent. The new hair from all grafts from back to belly (10 cases) grew conspicuously more rapidly than those from the surrounding shaven region and reached the characteristic length and color of back hair. All grafts from belly to back (6 cases) produced short, light-colored belly hair, markedly in contrast with the surroundings. The differences remained unchanged as long as observed (at least six months). The differences of the hair of the two regions are so striking as to leave no doubt of the persistence of the hair characters.

After having examined a number of grafts, we noted the constancy with which the direction of the graft hair agreed with the direction of the host hair. Since hair direction in the guinea-pig is affected by well-known gene differences, the question of maintenance of direction in a new environment is of interest.

Unfortunately, we had failed to record the orientation of the graft in the operations already performed, but thought it improbable that the graft should, in every case, have been oriented so that the direction of the hair coincided with that of the host. In the experiments that followed, we reversed the grafts 180° so that the hair of the graft would be growing in opposite direction to that of the surroundings. In these cases, we kept the surrounding area clipped so that there would not be the factor of pressure to influence the growth of the graft hair.

The results obtained are not strongly in favor of either conformity to the original direction, or regulation to the new environment. The tendency seemed to be for the hair to stand straight up. There were two cases out of the eighteen that showed definite regulation. One was a transplant of red skin to a black area in which the transplant was reversed 180°. The other case was a transplant of smooth, belly hair of a rough agouti animal to the region of a rosette on the back, the transplant being reversed 180°. There was a marked tendency for the hair of the graft to be arranged in a whorl to conform to the direction of the surrounding hair. In the sixteen other cases in which the graft was reversed, the hair either stood almost erect or exhibited a slight tendency toward its original direction.

The results of these experiments definitely indicate the persistence of color pattern and length of guinea-pig hair following autoplastic transplantation. The question of the maintenance of direction of hair growth is not definitely settled, two cases out of eighteen indicate regulation, the others exhibiting a tendency for the hair to stand erect rather than to show either persistence or regulation.[1]

The tendency toward persistence of the characters of the graft seems to indicate that the differences in these characters are determined entirely by permanent cell differentiation, chromosomal or otherwise. It is to be noted that these grafts involve transplantation of two different types of pattern, one type being highly irregular and the other regular. The irregular patterns are those produced by the spotting factors *s* and e^p, the spots of the different colors being very irregular in size and position on the body. The pattern of the agouti animals, on the other hand, is quite regular, the markedly different patterns of back and belly being bounded by a sharply defined, symmetrically placed line.

It is tempting to suppose that the persistent differentiation produced by the spotting factors is due to somatic mutation. If spotting effects are due to controlled somatic mutation, these

[1] Since this paper was written, Trotter and Dawson have published an account of experiments in which they reversed areas of guinea-pig skin, *in situ*, and found that in every case the graft hair maintained its original direction, even though the surrounding hair was allowed to grow. We have no explanation to offer for the partial disagreement of our results.

results suggest that such mutations are irreversible changes not affected by later correlative influences.

The regular pattern of the agouti animals has also been shown to be persistent. The manner in which the genetic differentiation of the two regions is brought about is unknown, the data indicating merely that the regional differences, both in color and length, are permanent within the skin itself. If somatic mutation is the correct interpretation of the differentiation in this case, it must be much more completely under control of the developmental pattern than in the cases of the irregular piebald and tortoise shell patterns.

C. H. SEEVERS
DONALD A. SPENCER

UNIVERSITY OF CHICAGO

LITERATURE CITED

Carnot, P., and Mlle. Cl. Deflandre
1896. *Compt. rend. Soc. de biol.*, Par., 10s: 178–180.
Danforth, C. H., and F. Foster
1929. *Jour. Exp. Zool.*, 52: 443–470.
Loeb, L.
1897. *Arch. f. Entw.-mech.* 6: 1–44.
Trotter, Mildred, and Helen Dawson
1931. *Anat. Rec.*, 50: 193–196.
Wright, S.
1927. *Genetics*, 12: 530–569.

AUSTRALIAN FRESH-WATER FISHES

THE few distinctive fresh-water fishes of Australia are sufficiently unlike them to have, at first thought, little place in consideration of those of the other major modern land masses. The dominant Ostariophycine group (characins, catfish, carps) is represented in Australian fresh waters by a very few species only, belonging to two estuarine groups of catfishes. We have in Australian fresh waters the relic primitive *Galaxias* and *Ceratodus* (lung-fish), with relatives in each of the southern continents, from which one may argue southern land-connection and distribution for such forms at some point in pretertiary time. We have a genus of the typically marine Apogonidae, *Glossamia,* adapted to fresh water, and in Tasmania the peculiar cod-like blackfish, *Gadopsis,* without known relationships.

Of particular interest are small fishes allied to the marine silversides (Atherinidae), which, lacking normal competitors, have

seemingly entered fresh water and there developed several peculiar divergent forms. One of these, *Melanotaenia,* suggests labyrinth fishes of southern Asia in body and fin form, and has pungent perch-like spines in dorsal and anal fin. Another, the blue-eye, *Pseudomugil,* is suggestive of certain carps and characins in appearance.

If one concede that Australia was separated from Asia throughout the Tertiary, absence of continental fresh-water groups there is evidence that these are of Tertiary origin and distribution, a conclusion already indicated on other grounds. That estuarine catfishes, established in Australian fresh waters with a comparatively open field, show no marked divergent specialization would indicate them to be a recent, not an early trend of catfishes, and to have played little part in the general world distribution of the catfish group by bridging areas of sea.

This discussion is incident to the examination of a small Australian collection, which Mr. Raven obtained for the museum several years ago, but which has only recently been studied in detail. We have found no previously published illustration of the handsome characteristically marked *Craterocephalus stercusmuscarum.* Incidentally, McCulloch's checklist of New South

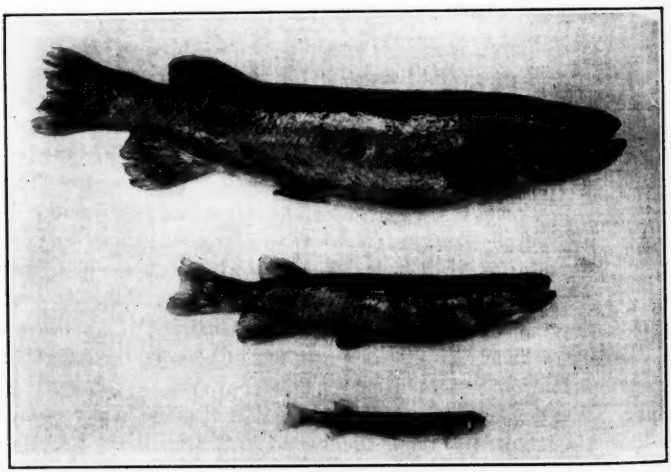

FIG. 1. Galaxias. Three stages of growth of specimens from the Arthur River, Tasmania.

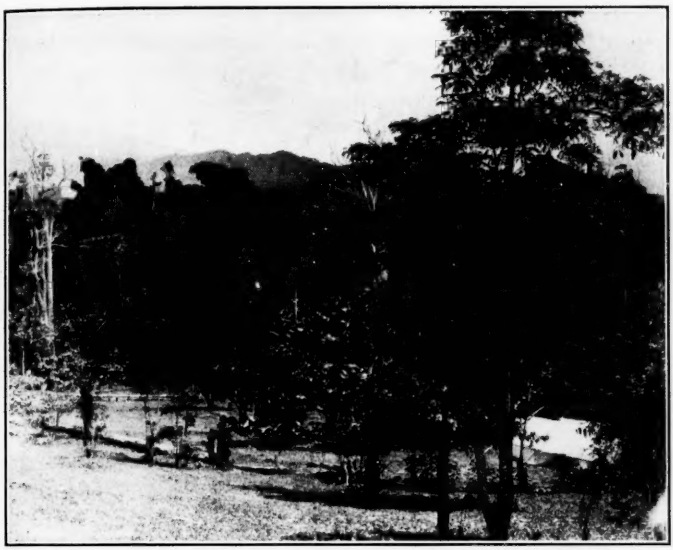

FIG. 2. Just beyond the tent is the little stream, a branch of Babinda Creek, from which the specimens were taken. In flood it covers the foreground to the center of the picture.

Wales fishes in the "Australian Zoologist" (1920–1922) is very fully illustrated, and a serviceable key to Australian fishes, both fresh-water and marine.

Most of the fishes comprising the above-mentioned collection were obtained in North Queensland, from a little stream arising on the slopes of Mount Bartle Frere, just south of the Bellenden Kerr Range, at a point where the stream emerges from densely forested foothills. About half a mile farther north this stream is confluent with Babinda Creek, which in turn is a tributary of the Russell River. All the fishes were secured by means of a minnow seine during the latter half of October, 1921.

The environment of this part of Australia has more in common with New Guinea than any other part of the continent. The Russell River system drains much of the coastal slope of the Cape York Peninsula between Cairns and Cardwell on the Pacific side. This region, eighty-five miles long by twenty miles wide, is the only part of Australia with a rainfall of more than 100 inches per annum.[1] Here, too, it is not very uncommon to record a rainfall of more than twenty inches in twenty-four hours.

[1] Taylor, Griffith, "The Australian Environment," p. 115. 1918.

This region of heavy rainfall is for the most part covered with tropical rainforest, the vegetation having much more in common with that of New Guinea than with immediately adjacent parts of Queensland. These conditions of rainfall and vegetation have apparently restricted many of the higher vertebrates to this limited area and kept still other widely distributed Australian forms out of this specialized zone. This region of heavy rain-

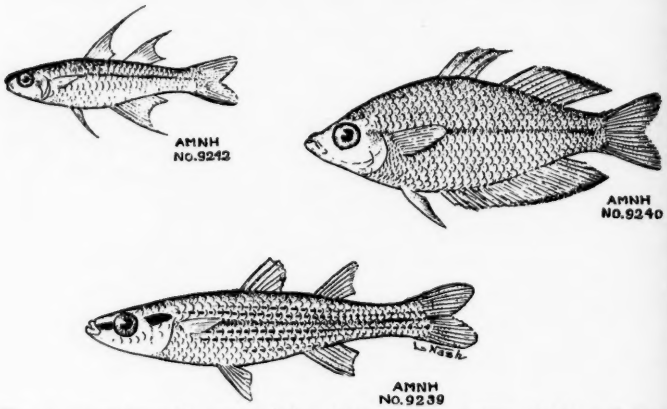

FIG. 3. *Pseudomugil* (upper left), *Melanotaenia* (upper right) and *Craterocephalus stercusmuscarum* (lower).

fall doubtlessly furnishes an abundance of food to the fishes of the streams, and it would be interesting to compare the forms inhabiting these swift streams of the well-watered area with those of streams of the drier parts of eastern Australia.

The atherinids in the collection were by far the most numerous fishes near Babinda and were commonly seen in small shoals, whereas the catfish were apparently not gregarious. The galaxias, which were obtained from the Arve and the Arthur Rivers of Tasmania, have somewhat the habits of trout but are much less active.

J. T. NICHOLS
H. C. RAVEN

THE AMERICAN MUSEUM
OF NATURAL HISTORY